XP-002128759

P.D. 03-1999
p. 248 = (1)

248P CHARACTERIZATION OF SIB-1757 AND SIB-1893: HIGHLY SELECTIVE ANTAGONISTS AT METABOTROPIC GLUTAMATE RECEPTOR SUBTYPE 5

M.A.Varney, N. Cosford, C. Jachec, S. Rao, A. Sacaan, E. Santori, [a]H. Allgeier, [a]F. Gasparini, [a]P. J. Flor, [a]R. Kuhn, S.D Hess, G.Veliçelebi & E. C. Johnson. SIBIA Neurosciences, Inc., La Jolla, CA 92037, USA & [a]Novartis Pharma AG, Nervous System Research, Basel, Switzerland.

Based on amino acid sequence identity, the eight identified metabotropic glutamate receptors (mGluRs) can be divided into three groups (I, II and III). Group I mGluRs includes both mGluR1 and mGluR5, and activation of these G-protein-coupled receptors stimulates phospholipase C. Understanding the role of group I mGluRs in normal physiology and pathophysiology has been hampered by the lack of potent and selective ligands for these receptor subtypes. Here we report the identification of structurally novel, highly selective mGluR5 antagonists.

We have previously reported the establishment of stable cells lines expressing recombinant human mGluR1b (hmGluR1b/L13-23-7 cells) and mGluR5a (hmGluR5a/L38-20 cells) (Daggett *et al.*, 1995; Lin *et al.*, 1997). These cell lines give robust increases in inositol phosphates (IP) and intracellular Ca^{2+} when activated by group I mGluR agonists such as dihydroxyphenylglycine (DHPG). The activity of compounds obtained from a random library of small molecules was evaluated on both cell lines using an automated high throughput screening system that detects changes in Ca^{2+} (Veliçelebi *et al.*, 1998). One compound, SIB-1757 (6-methyl-2-(phenylazo)-pyridin-3-ol), was identified as an antagonist at hmGluR5 with an IC_{50} of 0.4 (0.2, 0.7) μM (geometric mean, (lower, upper SD), N=5), and an IC_{50} >30 μM at hmGluR1b (N=5).

Testing of analogues of SIB-1757 led to the identification of an equipotent compound, SIB-1893 ((E)-6-methyl-2-styryl-pyridine). SIB-1893 selectively inhibited glutamate-stimulated Ca^{2+} signals at hmGluR5 with an IC_{50} of 0.3 (0.1, 0.6) μM (N=5), compared to an IC_{50} of >30 μM at hmGluR1b. The activities of SIB-1757 and SIB-1893 were evaluated at additional glutamate receptor subtypes. Using cAMP measurements, the agonist and antagonist potencies of SIB-1757 and SIB-1893 at group II and III mGluRs were >30 μM at recombinant hmGluR2, hmGluR4, hmGluR6, hmGluR7 and hmGluR8 (N=4-6).

Ca^{2+} measurements were used to determine the agonist and antagonist activities of SIB-1757 and SIB-1893 at recombinant AMPA receptors (hGluR1$_o$, hGluR2(Q)$_o$, hGluR3$_o$, hGluR4$_o$), kainate receptors (hGluR5 and hGluR6) and NMDA receptors (hNR1/2A and hNR1/2D). The agonist and antagonist potencies of SIB-1757 and SIB-1893 were >30 μM at these ionotropic glutamate receptors (N=3).

The potency of these compounds was examined in rat neonatal (8-12d) brain regions. In striatal tissue slices, the group I selective agonist DHPG (10 μM) evoked an increase in IP accumulation. SIB-1757 inhibited 68 ± 9 % of the DHPG-induced IP accumulation with an IC_{50} of 3.3 (1.5, 7.3) μM (N=3). In contrast, in the cerebellum, a brain region that has a low expression of mGluR5 and a higher expression of mGluR1 (Testa *et al.*, 1994), 100 μM SIB-1757 inhibited a maximum of 4 ± 10 % of DHPG-induced IP accumulation.

In conclusion, this is the first report of potent, subtype-selective antagonists at mGluR5 that can markedly discriminate between mGluR5 and mGluR1. SIB-1757 and SIB-1893, and further analogues (see Gasparini *et al.*, this meeting) are valuable tools for investigating the role of mGluR5 in models of pain (see Bowes *et al.*, this meeting) and CNS disorders.

Daggett, L.P., Sacaan, A.I., Akong, M. *et al.*, (1995) *Neuropharmacol* 34, 871-886
Lin, F.F., Varney, M.A., Sacaan, A.I. *et al.*, (1997) *Neuropharmacol* 36, 917-931
Testa, C., Standaert, D.G., Young, A.B. & Penney, J.B. (1994) *J. Neurosci.* 14, 3005-3018
Veliçelebi, G., Stauderman, K. A., Varney, M.A. *et al.*, (1998) *Meth. Enzymol.* 294, 20-47
Gasparini, F., Lingenhoehl, K., Flor, P., *et al., This meeting.*
Bowes, M., Panesar, M., Gentry, C., *et al., This meeting.*

Translation

PATENT COOPERATION TREATY

10/088350

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

10/088350

Applicant's or agent's file reference BET 00/0866	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/FR00/02577	International filing date (*day/month/year*) 15 September 2000 (15.09.00)	Priority date (*day/month/year*) 17 September 1999 (17.09.99)
International Patent Classification (IPC) or national classification and IPC A23C 9/1123		
Applicant TEXEL		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14 March 2001 (14.03.01)	18 January 2002 (18.01.2002)
Name and mailing address of the IPEA/EP Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

national application No.
PCT/FR00/02577

I. Basis of the report

1. This report has been drawn on the basis of (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

☒ the international application as originally filed.

☐ the description, pages 1-20, as originally filed,
pages _____, filed with the demand,
pages _____, filed with the letter of _____,
pages _____, filed with the letter of _____.

☐ the claims, Nos. _____, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. 1-9, filed with the letter of 27 November 2001 (27.11.2001),
Nos. _____, filed with the letter of _____.

☐ the drawings, sheets/fig 1-15, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig _____, filed with the letter of _____,
sheets/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/fig _____

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

V. **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-9	**YES**
	Claims		**NO**
Inventive step (IS)	Claims	1-9	**YES**
	Claims		**NO**
Industrial applicability (IA)	Claims	1-9	**YES**
	Claims		**NO**

2. Citations and explanations

The following documents are referred to:

D1: W. TINSON: "Metabolism of streptococcus thermophilus", THE AUSTRALIAN JOURNAL OF DAIRY TECHNOLOGY, Vol. 37, N° 1, 1982, pages 17-21, XP002141061, cited in the application

D2: B. BIANCHI SALVADORI: "Characteristics of some streptococcus thermophilus strains for the preparation of starters dehydrated for direct inoculation in cheese-vats", SCIENZA E TECNICA LATTIERO-CASEARIA, Vol. 34, N° 4, 1983, pages 227-248, XP000920986

D3: A. ZOURARI: "Caractérisation de bactéries lactiques thermophiles isolées de yaourts artisanaux grecs", LE LAIT, Vol. 77, N° 4, 1991, pages 445-461, XP000921064.

The new set of Claims 1-9 filed on 27-11-01 satisfies the requirements of PCT Article 34(2)(b). Claim 9 has been amended so as to specify that the selection of the mutant strains is based on their acidifying properties, which are different from those of the parental strains, and involves comparing acidification kinetics. Support for this amendment can be found on page 13, lines 12-14, of the description,

and on page 16, lines 6-19.

Claims 1-8 define the use and method of implementing a *Streptococcus thermophilus (ur-)* strain in the manufacture of cheeses or fermented dairy products, in order to obtain acidification kinetics which are not dependent on the milk constituent content. Claim 9 defines a method for selecting a *Streptococcus thermophilus (ur-)* strain based on acidification kinetics. None of the prior art documents D1-D3 discloses a use or method as claimed, or contains any indication of the subject matter of Claims 1-9. The subject matter of Claims 1-9 is therefore novel and inventive.

D1 examines not the acidification kinetics but the CO_2 production of *Streptococcus thermophilus (ur-)* strains. This document does, however, contain indications of the rate of acidification (see page 18, right-hand column, first paragraph) which, by contrast, show no difference in relation to the parental strains (ur+).

D2 concerns the selection of *Streptococcus thermophilus* strains which are advantageous in cheese manufacturing. In particular, this document discloses the use of (ur+) strains all of which display urease activity, except for one (strain 8A, Table 4). However, the acidification properties of the urease-deficient strain 8A are not studied separately from those of the other ur+ strains. Strain 8A was therefore not selected specifically for the fact that it does not hydrolyse urea. Moreover, D2 provides no indication that the acidification properties of strain 8A are independent of the composition of the milk.

D3 concerns the characterisation of *S. thermophilus* strains all displaying urease activity and contains no indication prompting a person skilled in the art to select exclusively from strains displaying no urease activity or reduced urease activity.

(12) DEMANDE INTERNATIONALE PUBLIÉE EN VERTU DU TRAITÉ DE COOPÉRATION EN MATIÈRE DE BREVETS (PCT)

(19) Organisation Mondiale de la Propriété Intellectuelle
Bureau international

(43) Date de la publication internationale
5 avril 2001 (05.04.2001)

PCT

(10) Numéro de publication internationale
WO 01/22828 A1

(51) Classification internationale des brevets[7]: A23C 9/123, 19/032

(21) Numéro de la demande internationale:
PCT/FR00/02577

(22) Date de dépôt international:
15 septembre 2000 (15.09.2000)

(25) Langue de dépôt: français

(26) Langue de publication: français

(30) Données relatives à la priorité:
99/11677 17 septembre 1999 (17.09.1999) FR

(71) Déposants *(pour tous les États désignés sauf US)*: TEXEL [FR/FR]; Zone d'activités de Buxières, F-86220 Dange Saint Romain (FR). INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE [FR/FR]; 147, rue de l'Université, F-75338 Paris Cedex 07 (FR).

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(74) Mandataire: JACOBSON, Claude; Cabinet Lavoix, 2, place d'Estienne d'Orves, F-75441 Paris Cedex 09 (FR).

(81) États désignés *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) États désignés *(régional)*: brevet ARIPO (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), brevet eurasien (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), brevet européen (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), brevet OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Publiée:
— *Avec rapport de recherche internationale.*

En ce qui concerne les codes à deux lettres et autres abréviations, se référer aux "Notes explicatives relatives aux codes et abréviations" figurant au début de chaque numéro ordinaire de la Gazette du PCT.

(54) Title: USE OF STRAINS OF STREPTOCOCCUS THERMOPHILUS WHICH ARE INCAPABLE OF HYDROLYZING UREA IN DAIRY PRODUCTS

(54) Titre: UTILISATION DE SOUCHES *STREPTOCOCCUS THERMOPHILUS* INCAPABLES D'HYDROLYSER L'UREE DANS DES PRODUITS LAITIERS

(57) Abstract: The invention relates to the use of at least one strain of *Streptococcus thermophilus* which is at least partially, preferably totally, incapable of hydrolyzing urea in the manufacture of cheese or fermented dairy products such as yoghurts in order to obtain an acidification kinetic which is independent from the content of various components of milk.

(57) Abrégé: Cette invention concerne l'utilisation d'au moins une souche *Streptococcus thermophilus* au moins partiellement, de préférence totalement, incapable d'hydrolyser l'urée, lors de la fabrication de fromages ou de produits laitiers fermentés tels que des yaourts, pour obtenir une cinétique d'acidification substantiellement indépendante de la teneur en divers composants du lait.

WO 01/22828 A1

CLAIMS

1. Use of at least one strain of *Streptococcus thermophilus* which is at least partially, preferably totally, incapable of hydrolyzing urea, during the manufacture of cheeses or fermented dairy products such as yoghurts, in order to obtain an acidification kinetic which is substantially independent of the content of the milk in terms of its constituents.

2. Use according to Claim 1, in which the acidification kinetic is substantially independent of the urea content of the milk.

3. Use according to Claim 1, in which the acidification kinetic of the milk is substantially independent of the nickel or cobalt content of the milk.

4. Use according to one of the preceding claims, in which the acidification kinetic of the milk does not exhibit any temporary slowing down.

5. Use according to any one of the preceding claims, in which the *Streptococcus thermophilus* strain is the strain 298-K registered at the CNCM under the number I-2311.

6. Use according to any one of Claims 1 to 4, in which the *Streptococcus thermophilus* strain is the strain 298-10 registered at the CNCM under the number I-2312.

7. Method for obtaining, during the manufacture of cheeses or fermented dairy products such as yoghurts, an acidification kinetic which is substantially independent of the content of the milk in terms of its constituents, in which there is incorporated with the milk at least one strain of

Streptococcus thermophilus which is at least partially, preferably totally, incapable of hydrolyzing urea.

8. Method according to Claim 7, in which there is incorporated with the milk at least one mutant strain of *Streptococcus thermophilus* which is at least partially, preferably totally, incapable of hydrolyzing urea, at a seeding rate lower than the seeding rate used for the parent strain of *Streptococcus thermophilus* capable of hydrolyzing urea.

9. Method of selecting *Streptococcus thermophilus* strains useful during the manufacture of cheeses or fermented dairy products, in which mutant strains of *Streptococcus thermophilus* which are at least partially, preferably totally, incapable of hydrolyzing urea, allowing an acidification kinetic to be obtained which is substantially independent of the content of the milk in terms of its constituents, are selected for their ability to acidify a milk according to acidification kinetics which are variable compared with the acidification kinetics of the parent strains.

TRAITE DE COOPERATION EN MATIERE DE BREVETS

10/088350

PCT

RAPPORT D'EXAMEN PRELIMINAIRE INTERNATIONAL

(article 36 et règle 70 du PCT)

Référence du dossier du déposant ou du mandataire BET 00/0866	**POUR SUITE A DONNER** voir la notification de transmission du rapport d'examen préliminaire international (formulaire PCT/IPEA/416)	
Demande internationale n° PCT/FR00/02577	Date du dépot international *(jour/mois/année)* 15/09/2000	Date de priorité *(jour/mois/année)* 17/09/1999
Classification internationale des brevets (CIB) ou à la fois classification nationale et CIB A23C9/123		
Déposant TEXEL		

1. Le présent rapport d'examen préliminaire international, établi par l'administaration chargée de l'examen préliminaire international, est transmis au déposant conformément à l'article 36.

2. Ce RAPPORT comprend 4 feuilles, y compris la présente feuille de couverture.

   ☒ Il est accompagné d'ANNEXES, c'est-à-dire de feuilles de la description, des revendications ou des dessins qui ont été modifiées et qui servent de base au présent rapport ou de feuilles contenant des rectifications faites auprès de l'administration chargée de l'examen préliminaire international (voir la règle 70.16 et l'instruction 607 des Instructions administratives du PCT).

   Ces annexes comprennent 2 feuilles.

3. Le présent rapport contient des indications relatives aux points suivants:

   - I ☒ Base du rapport
   - II ☐ Priorité
   - III ☐ Absence de formulation d'opinion quant à la nouveauté, l'activité inventive et la possibilité d'application industrielle
   - IV ☐ Absence d'unité de l'invention
   - V ☒ Déclaration motivée selon l'article 35(2) quant à la nouveauté, l'activité inventive et la possibilité d'application industrielle; citations et explications à l'appui de cette déclaration
   - VI ☐ Certains documents cités
   - VII ☐ Irrégularités dans la demande internationale
   - VIII ☐ Observations relatives à la demande internationale

Date de présentation de la demande d'examen préliminaire internationale	Date d'achèvement du présent rapport
14/03/2001	18.01.2002
Nom et adresse postale de l'administration chargée de l'examen préliminaire international: Office européen des brevets D-80298 Munich Tél. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Fonctionnaire autorisé Vermeulen, S N° de téléphone +49 89 2399 7520

Formulaire PCT/IPEA/409 (feuille de couverture) (janvier 1994)

RAPPORT D'EXAMEN PRÉLIMINAIRE INTERNATIONAL

Demande int mationale n° PCT/FR00/02577

I. Base du rapport

1. En ce qui concerne les **éléments** de la demande internationale (*les feuilles de remplacement qui ont été remises à l'office récepteur en réponse à une invitation faite conformément à l'article 14 sont considérées dans le présent rapport comme "initialement déposées" et ne sont pas jointes en annexe au rapport puisqu'elles ne contiennent pas de modifications (règles 70.16 et 70.17)*):

Description, pages:

1-20 version initiale

Revendications, N°:

1-9 reçue(s) avec télécopie du 27/11/2001

Dessins, feuilles:

1-15 version initiale

2. En ce qui concerne la **langue**, tous les éléments indiqués ci-dessus étaient à la disposition de l'administration ou lui ont été remis dans la langue dans laquelle la demande internationale a été déposée, sauf indication contraire donnée sous ce point.

   Ces éléments étaient à la disposition de l'administration ou lui ont été remis dans la langue suivante: , qui est :

   - ☐ la langue d'une traduction remise aux fins de la recherche internationale (selon la règle 23.1(b)).
   - ☐ la langue de publication de la demande internationale (selon la règle 48.3(b)).
   - ☐ la langue de la traduction remise aux fins de l'examen préliminaire internationale (selon la règle 55.2 ou 55.3).

3. En ce qui concerne les **séquences de nucléotides ou d'acide aminés** divulguées dans la demande internationale (le cas échéant), l'examen préliminaire internationale a été effectué sur la base du listage des séquences :

   - ☐ contenu dans la demande internationale, sous forme écrite.
   - ☐ déposé avec la demande internationale, sous forme déchiffrable par ordinateur.
   - ☐ remis ultérieurement à l'administration, sous forme écrite.
   - ☐ remis ultérieurement à l'administration, sous forme déchiffrable par ordinateur.
   - ☐ La déclaration, selon laquelle le listage des séquences par écrit et fourni ultérieurement ne va pas au-delà de la divulgation faite dans la demande telle que déposée, a été fournie.
   - ☐ La déclaration, selon laquelle les informations enregistrées sous déchiffrable par ordinateur sont identiques à celles du listages des séquences Présenté par écrit, a été fournie.

4. Les modifications ont entraîné l'annulation :

- ☐ de la description, pages :
- ☐ des revendications, n°s :
- ☐ des dessins, feuilles :

5. ☐ Le présent rapport a été formulé abstraction faite (de certaines) des modifications, qui ont été considérées comme allant au-delà de l'exposé de l'invention tel qu'il a été déposé, comme il est indiqué ci-après (règle 70.2(c)) :

   (Toute feuille de remplacement comportant des modifications de cette nature doit être indiquée au point 1 et annexée au présent rapport)

6. Observations complémentaires, le cas échéant :

V. Déclaration motivée selon l'article 35(2) quant à la nouveauté, l'activité inventive et la possibilité d'application industrielle; citations et explications à l'appui de cette déclaration

1. Déclaration

Nouveauté	Oui :	Revendications	1-9
	Non :	Revendications	
Activité inventive	Oui :	Revendications	1-9
	Non :	Revendications	
Possibilité d'application industrielle	Oui :	Revendications	1-9
	Non :	Revendications	

2. Citations et explications
   voir feuille séparée

Conc rnant le point V

Déclaration motivée selon l'article 35(2) quant à la nouveauté, l'activité inventive et la possibilité d'application industrielle; citations et explications à l'appui de cette déclaration

Il est fait référence aux documents suivants:

D1: W. TINSON: 'Metabolism of streptococcus thermophilus' THE AUSTRALIAN JOURNAL OF DAIRY TECHNOLOGY, vol. 37, no. 1, 1982, pages 17-21, XP002141061 cité dans la demande

D2: B. BIANCHI SALVADORI: 'Characteristics of some streptococcus thermophilus strains for the preparation of starters dehydrated for direct inoculation in cheese-vats' SCIENZA E TECNICA LATTIERO-CASEARIA, vol. 34, no. 4, 1983, pages 227-248, XP000920986

D3: A. ZOURARI: 'Caractérisation de bactéries lactiques thermophiles isolées de yaourts artisanaux grecs' LE LAIT, vol. 77, no. 4, 1991, pages 445-461, XP000921064

Le nouveau jeu de revendications 1-9 déposé le 27-11-01 remplit les conditions enoncées à l'article 34(2)b PCT. La revendication 9 a été modifiée de manière à préciser que la sélection des souches mutantes est basée sur leurs propriétés acidifiantes différentes de celles des souches parentales, par comparaison des cinétiques d'acidification. Le support pour cet amendement se trouve page 13, lignes 12-14 de la description, et page 16, lignes 6-19.

Les revendications 1-8 definissent l'utilisation et le procédé de mise en oeuvre d'une souche *Streptococcus thermophilus (ur-)* dans la fabrication de fromages ou de produits laitiers fermentés pour obtenir une cinétique d'acidification indépendante de la teneur du lait en ses composants. La revendication 9 définit un procédé de sélection d'une souche *Streptococcus thermophilus (ur-)* basé sur la cinétique d'acidification. Aucun des documents d'art antérieur D1-D3 ne divulgue une utilisation ou un procédé tel que revendiqué ni ne contient d'indications qui pourraient mener à l'objet des revendications 1-9. L'objet des revendications 1-9 est par conséquent nouveau et inventif.

D1 n'étudie pas la cinétique d'acidification mais la production de CO_2 de souches *Streptococcus thermophilus (ur-)*. Le document contient malgré tout des indications concernant la vitesse d'acidification (cf. page 18, colonne de droite, premier paragraphe), qui au contraire ne présente aucune différence par rapport aux souches parentales (ur+).

D2 concerne la sélection de souches Streptococcus thermophilus avantageuses dans la fabrication de fromages. Le document divulgue surtout l'emploi de souches (ur+) qui toutes présentent une activité uréasique, sauf une (souche 8A, tableau 4). Les qualités d'acidification de la souche 8A déficiente en uréase ne sont toutefois pas étudiées indépendamment de celles des autres souches ur+. La souche 8A n'est donc pas particulièrement sélectionnée pour son absence d'hydrolyse de l'urée. Par ailleurs, le document D2 n'indique nullement que les qualités d'acidification de la souche 8A sont indépendantes de la composition du lait.

D3 a trait à la caractérisation de souches *S. thermophilus* possédant toutes une activité uréasique et ne contient pas d'indication qui mènerait l'homme du métier à la sélection parmi des seules souches déficientes en activité uréasique ou présentant une activité uréasique réduite.

TRAITE DE COOPERATION EN MATIERE DE BREVETS

Expéditeur : le BUREAU INTERNATIONAL

PCT

NOTIFICATION RELATIVE A LA PRESENTATION OU A LA TRANSMISSION DU DOCUMENT DE PRIORITE

(instruction administrative 411 du PCT)

Destinataire:

JACOBSON, Claude
Cabinet Lavoix
2, place d'Estienne d'Orves
F-75441 Paris Cedex 09
FRANCE

Date d'expédition (jour/mois/année) 01 novembre 2000 (01.11.00)	
Référence du dossier du déposant ou du mandataire BET 00/0866	**NOTIFICATION IMPORTANTE**
Demande internationale no PCT/FR00/02577	Date du dépôt international (jour/mois/année) 15 septembre 2000 (15.09.00)
Date de publication internationale (jour/mois/année) Pas encore publiée	Date de priorité (jour/mois/année) 17 septembre 1999 (17.09.99)
Déposant TEXEL etc	

1. La date de réception (sauf lorsque les lettres "NR" figurent dans la colonne de droite) par le Bureau international du ou des documents de priorité correspondant à la ou aux demandes énumérées ci-après est notifiée au déposant. Sauf indication contraire consistant en un astérisque figurant à côté d'une date de réception, ou les lettres "NR", dans la colonne de droite, le document de priorité en question a été présenté ou transmis au Bureau international d'une manière conforme à la règle 17.1.a) ou b).
2. Ce formulaire met à jour et remplace toute notification relative à la présentation ou à la transmission du document de priorité qui a été envoyée précédemment.
3. Un astérisque(*) figurant à côté d'une date de réception dans la colonne de droite signale un document de priorité présenté ou transmis au Bureau international mais de manière non conforme à la règle 17.1.a) ou b). Dans ce cas, l'attention du déposant est appelée sur la règle 17.1.c) qui stipule qu'aucun office désigné ne peut décider de ne pas tenir compte de la revendication de priorité avant d'avoir donné au déposant la possibilité de remettre le document de priorité dans un délai raisonnable en l'espèce.
4. Les lettres "NR" figurant dans la colonne de droite signalent un document de priorité que le Bureau international n'a pas reçu ou que le déposant n'a pas demandé à l'office récepteur de préparer et de transmettre au Bureau international, conformément à la règle 17.1.a) ou b), respectivement. Dans ce cas, l'attention du déposant est appelée sur la règle 17.1.c) qui stipule qu'aucun office désigné ne peut décider de ne pas tenir compte de la revendication de priorité avant d'avoir donné au déposant la possibilité de remettre le document de priorité dans un délai raisonnable en l'espèce.

Date de priorité	Demande de priorité n°	Pays, office régional ou office récepteur selon le PCT	Date de réception du document de priorité
17 sept 1999 (17.09.99)	99/11677	FR	17 octo 2000 (17.10.00)

Bureau international de l'OMPI
34, chemin des Colombettes
1211 Genève 20, Suisse

no de télécopieur (41-22) 740.14.35

Fonctionnaire autorisé:

Philippe Bécamel

no de téléphone (41-22) 338.83.38

Formulaire PCT/IB/304 (juillet 1998)

003625753

TRAITE DE COOPERATION EN MATIERE DE BREVETS

Expéditeur: L'ADMINISTRATION CHARGÉE DE L'EXAMEN PRELIMINAIRE INTERNATIONAL

Destinataire:

LE GUEN Gerard
CABINET LAVOIX
2, place d'Estienne d'Orves
75441 Paris Cédex 09
FRANCE

PCT

NOTIFICATION DE TRANSMISSION DU RAPPORT D'EXAMEN PRELIMINAIRE INTERNATIONAL

(règle 71.1 du PCT)

DI 18.03.02

Date d'expédition *(jour/mois/année)*	18.01.2002
Référence du dossier du déposant ou du mandataire: BET 00/0866	NOTIFICATION IMPORTANTE

Demande internationale No.	Date du dépot international *(jour/mois/année)*	Date de priorité *(jour/mois/année)*
PCT/FR00/02577	15/09/2000	17/09/1999

Déposant: TEXEL 99 11672

1. Il est notifié au déposant que l'administration chargée de l'examen préliminaire international a établi le rapport d'examen préliminaire international pour la demande internationale et le lui transmet ci-joint, accompagné, le cas échéant, de ces annexes.

2. Une copie du présent rapport et, le cas échéant, de ses annexes est transmise au Bureau international pour communication à tous les offices élus.

3. Si tel ou tel office élu l'exige, le Bureau international établira une traduction en langue anglaise du rapport (à l'exclusion des annexes de celui-ci) et la transmettra aux offices intéressés.

4. **RAPPEL**

Pour aborder la phase nationale auprès de chaque office élu, le déposant doit accomplir certains actes (dépôt de traduction et paiement des taxes nationales) dans le délai de 30 mois à compter de la date de priorité (ou plus tard pour ce qui concerne certains offices) (article 39.1) (voir aussi le rappel envoyé par le Bureau international dans le formulaire PCT/IB/301).

Lorsqu'une traduction de la demande internationale doit être remise à un office élu, elle doit comporter la traduction de toute annexe du rapport d'examen préliminaire international. Il appartient au déposant d'établir la traduction en question et de la remettre directement à chaque office élu intéressé.

Pour plus de précisions en ce qui concerne les délais applicables et les exigences des offices élus, voir le Volume II du Guide du déposant du PCT.

Nom et adresse postale de l'adminstration chargée de l'examen préliminaire international	Fonctionnaire autorisé
Office européen des brevets D-80298 Munich Tél. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Götz, K Tél.+49 89 2399-7381

Formulaire PCT/IPEA/416 (juillet 1992)

10/088350

PCT

REQUÊTE

Le soussigné requiert que la présente demande internationale soit traitée conformément au Traité de coopération en matière de brevets.

Réservé à l'office récepteur

Demande internationale n°

Date du dépôt international

Nom de l'office récepteur et "Demande internationale PCT"

Référence du dossier du déposant ou du mandataire *(facultatif)* *(12 caractères au maximum)* BET 00/0866

Cadre n° I TITRE DE L'INVENTION " Utilisation de souches Streptococcus thermophilus incapables d'hydrolyser l'urée pour maîtriser les cinétiques d'acidification du lait dans l'industrie laitière ".

Cadre n° II DÉPOSANT

Nom et adresse : *(Nom de famille suivi du prénom; pour une personne morale, désignation officielle complète. L'adresse doit comprendre le code postal et le nom du pays. Le pays de l'adresse indiquée dans ce cadre est l'État où le déposant a son domicile si aucun domicile n'est indiqué ci-dessous.)*

TEXEL
Zone d'activités de Buxières
86220 DANGE ST ROMAIN FRANCE

☐ Cette personne est aussi inventeur.

n° de téléphone

n° de télécopieur

n° de téléimprimeur

Nationalité (nom de l'État) : FR

Domicile (nom de l'État) : FR

Cette personne est déposant pour : ☐ tous les États désignés ☒ tous les États désignés sauf les États-Unis d'Amérique ☐ les États-Unis d'Amérique seulement ☐ les États indiqués dans le cadre supplémentaire

Cadre n° III AUTRE(S) DÉPOSANT(S) OU (AUTRE(S)) INVENTEUR(S)

Nom et adresse : *(Nom de famille suivi du prénom; pour une personne morale, désignation officielle complète. L'adresse doit comprendre le code postal et le nom du pays. Le pays de l'adresse indiquée dans ce cadre est l'État où le déposant a son domicile si aucun domicile n'est indiqué ci-dessous.)*

INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE
147 rue de l'Université
75338 PARIS CEDEX 07 FRANCE

Cette personne est :

☒ déposant seulement

☐ déposant et inventeur

☐ inventeur seulement *(Si cette case est cochée, ne pas remplir la suite.)*

Nationalité (nom de l'État) : FR

Domicile (nom de l'État) : FR

Cette personne est déposant pour : ☐ tous les États désignés ☒ tous les États désignés sauf les États-Unis d'Amérique ☐ les États-Unis d'Amérique seulement ☐ les États indiqués dans le cadre supplémentaire

☒ D'autres déposants ou inventeurs sont indiqués sur une feuille annexe.

Cadre n° IV MANDATAIRE OU REPRÉSENTANT COMMUN; OU ADRESSE POUR LA CORRESPONDANCE

La personne dont l'identité est donnée ci-dessous est/a été désignée pour agir au nom du ou des déposants auprès des autorités internationales compétentes, comme: ☒ mandataire ☐ représentant commun

Nom et adresse : *(Nom de famille suivi du prénom; pour une personne morale, désignation officielle complète. L'adresse doit comprendre le code postal et le nom du pays.)*

JACOBSON Claude
CABINET LAVOIX
2, Place d'Estienne d'Orves
75441 PARIS CEDEX 09 FRANCE

n° de téléphone
01 53 20 14 20

n° de télécopieur
01 48 74 54 56

n° de téléimprimeur

☐ **Adresse pour la correspondance** : cocher cette case lorsque aucun mandataire ni représentant commun n'est/n'a été désigné et que l'espace ci-dessus est utilisé pour indiquer une adresse spéciale à laquelle la correspondance doit être envoyée.

PCT 154/130700

Suite du cadre n° III AUTRE(S) DÉPOSANT(S) OU (AUTRE(S)) INVENTEUR(S)

Si aucun des sous-cadres suivants n'est utilisé, cette feuille ne doit pas être incluse dans la requête.

Nom et adresse : *(Nom de famille suivi du prénom; pour une personne morale, désignation officielle complète. L'adresse doit comprendre le code postal et le nom du pays. Le pays de l'adresse indiquée dans ce cadre est l'État où le déposant a son domicile si aucun domicile n'est indiqué ci-dessous.)*

SEPULCHRE Anne-Marie
11, rue Moreau Chaumien
37550 SAINT-AVERTIN FRANCE

Cette personne est :
- [ ] déposant seulement
- [X] déposant et inventeur
- [ ] inventeur seulement *(Si cette case est cochée, ne pas remplir la suite.)*

Nationalité (nom de l'État) : FR

Domicile (nom de l'État) : FR

Cette personne est déposant pour :
- [ ] tous les États désignés
- [ ] tous les États désignés sauf les États-Unis d'Amérique
- [X] les États-Unis d'Amérique seulement
- [ ] les États indiqués dans le cadre supplémentaire

Nom et adresse : *(Nom de famille suivi du prénom; pour une personne morale, désignation officielle complète. L'adresse doit comprendre le code postal et le nom du pays. Le pays de l'adresse indiquée dans ce cadre est l'État où le déposant a son domicile si aucun domicile n'est indiqué ci-dessous.)*

MONNET Christophe
69, rue Jacques Durand
78370 PLAISIR FRANCE

Cette personne est :
- [ ] déposant seulement
- [X] déposant et inventeur
- [ ] inventeur seulement *(Si cette case est cochée, ne pas remplir la suite.)*

Nationalité (nom de l'État) : FR

Domicile (nom de l'État) : FR

Cette personne est déposant pour :
- [ ] tous les États désignés
- [ ] tous les États désignés sauf les États-Unis d'Amérique
- [X] les États-Unis d'Amérique seulement
- [ ] les États indiqués dans le cadre supplémentaire

Nom et adresse : *(Nom de famille suivi du prénom; pour une personne morale, désignation officielle complète. L'adresse doit comprendre le code postal et le nom du pays. Le pays de l'adresse indiquée dans ce cadre est l'État où le déposant a son domicile si aucun domicile n'est indiqué ci-dessous.)*

CORRIEU Georges
2, avenue des Combattants
78220 VIROFLAY FRANCE

Cette personne est :
- [ ] déposant seulement
- [X] déposant et inventeur
- [ ] inventeur seulement *(Si cette case est cochée, ne pas remplir la suite.)*

Nationalité (nom de l'État) : FR

Domicile (nom de l'État) : FR

Cette personne est déposant pour :
- [ ] tous les États désignés
- [ ] tous les États désignés sauf les États-Unis d'Amérique
- [X] les États-Unis d'Amérique seulement
- [ ] les États indiqués dans le cadre supplémentaire

Nom et adresse : *(Nom de famille suivi du prénom; pour une personne morale, désignation officielle complète. L'adresse doit comprendre le code postal et le nom du pays. Le pays de l'adresse indiquée dans ce cadre est l'État où le déposant a son domicile si aucun domicile n'est indiqué ci-dessous.)*

Cette personne est :
- [ ] déposant seulement
- [ ] déposant et inventeur
- [ ] inventeur seulement *(Si cette case est cochée, ne pas remplir la suite.)*

Nationalité (nom de l'État) :

Domicile (nom de l'État) :

Cette personne est déposant pour :
- [ ] tous les États désignés
- [ ] tous les États désignés sauf les États-Unis d'Amérique
- [ ] les États-Unis d'Amérique seulement
- [ ] les États indiqués dans le cadre supplémentaire

- [ ] D'autres déposants ou inventeurs sont indiqués sur une autre feuille annexe.

Cadre n° V DÉSIGNATION D'ÉTATS

Les désignations suivantes sont faites conformément à la règle 4.9.a) *(cocher les cases appropriées; une au moins doit l'être)* :

Brevet régional

☒ **AP** **Brevet ARIPO : GH** Ghana, **GM** Gambie, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **MZ** Mozambique, **SD** Soudan, **SL** Sierra Leone, **SZ** Swaziland, **TZ** République-Unie de Tanzanie, **UG** Ouganda, **ZW** Zimbabwe et tout autre État qui est un État contractant du Protocole de Harare et du PCT

☒ **EA** **Brevet eurasien : AM** Arménie, **AZ** Azerbaïdjan, **BY** Bélarus, **KG** Kirghizistan, **KZ** Kazakhstan, **MD** République de Moldova, **RU** Fédération de Russie, **TJ** Tadjikistan, **TM** Turkménistan et tout autre État qui est un État contractant de la Convention sur le brevet eurasien et du PCT

☒ **EP** **Brevet européen : AT** Autriche, **BE** Belgique, **CH** et **LI** Suisse et Liechtenstein, **CY** Chypre, **DE** Allemagne, **DK** Danemark, **ES** Espagne, **FI** Finlande, **FR** France, **GB** Royaume-Uni, **GR** Grèce, **IE** Irlande, **IT** Italie, **LU** Luxembourg, **MC** Monaco, **NL** Pays-Bas, **PT** Portugal, **SE** Suède et tout autre État qui est un État contractant de la Convention sur le brevet européen et du PCT

☒ **OA** **Brevet OAPI : BF** Burkina Faso, **BJ** Bénin, **CF** République centrafricaine, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroun, **GA** Gabon, **GN** Guinée, **GW** Guinée-Bissau, **ML** Mali, **MR** Mauritanie, **NE** Niger, **SN** Sénégal, **TD** Tchad, **TG** Togo et tout autre État qui est un État membre de l'OAPI et un État contractant du PCT *(si une autre forme de protection ou de traitement est souhaitée, le préciser sur la ligne pointillée)* .....

Brevet national *(si une autre forme de protection ou de traitement est souhaitée, le préciser sur la ligne pointillée)* :

☒ **AE** Émirats arabes unis
☒ **AG** Antigua-et-Barbuda
☒ **AL** Albanie .....
☒ **AM** Arménie .....
☒ **AT** Autriche .....
☒ **AU** Australie .....
☒ **AZ** Azerbaïdjan
☒ **BA** Bosnie-Herzégovine .....
☒ **BB** Barbade
☒ **BG** Bulgarie .....
☒ **BR** Brésil .....
☒ **BY** Bélarus .....
☒ **BZ** Belize
☒ **CA** Canada
☒ **CH** et **LI** Suisse et Liechtenstein
☒ **CN** Chine .....
☒ **CR** Costa Rica .....
☒ **CU** Cuba .....
☒ **CZ** République tchèque .....
☒ **DE** Allemagne .....
☒ **DK** Danemark .....
☒ **DM** Dominique
☒ **DZ** Algérie .....
☒ **EE** Estonie .....
☒ **ES** Espagne .....
☒ **FI** Finlande .....
☒ **GB** Royaume-Uni
☒ **GD** Grenade
☒ **GE** Géorgie .....
☒ **GH** Ghana .....
☒ **GM** Gambie
☒ **HR** Croatie .....
☒ **HU** Hongrie .....
☒ **ID** Indonésie
☒ **IL** Israël .....
☒ **IN** Inde .....
☒ **IS** Islande
☒ **JP** Japon .....
☒ **KE** Kenya .....
☒ **KG** Kirghizistan .....
☒ **KP** République populaire démocratique de Corée .....
☒ **KR** République de Corée .....
☒ **KZ** Kazakhstan .....
☒ **LC** Sainte-Lucie
☒ **LK** Sri Lanka
☒ **LR** Liberia
☒ **LS** Lesotho .....
☒ **LT** Lituanie
☒ **LU** Luxembourg
☒ **LV** Lettonie
☒ **MA** Maroc .....
☒ **MD** République de Moldova .....
☒ **MG** Madagascar .....
☒ **MK** Ex-République yougoslave de Macédoine .....
☒ **MN** Mongolie
☒ **MW** Malawi .....
☒ **MX** Mexique .....
☒ **MZ** Mozambique
☒ **NO** Norvège
☒ **NZ** Nouvelle-Zélande .....
☒ **PL** Pologne .....
☒ **PT** Portugal .....
☒ **RO** Roumanie
☒ **RU** Fédération de Russie .....
☒ **SD** Soudan
☒ **SE** Suède
☒ **SG** Singapour
☒ **SI** Slovénie .....
☒ **SK** Slovaquie .....
☒ **SL** Sierra Leone .....
☒ **TJ** Tadjikistan .....
☒ **TM** Turkménistan .....
☒ **TR** Turquie .....
☒ **TT** Trinité-et-Tobago .....
☒ **TZ** République-Unie de Tanzanie .....
☒ **UA** Ukraine .....
☒ **UG** Ouganda .....
☒ **US** États-Unis d'Amérique .....
☒ **UZ** Ouzbékistan .....
☒ **VN** Viet Nam .....
☒ **YU** Yougoslavie .....
☒ **ZA** Afrique du Sud .....
☒ **ZW** Zimbabwe .....

Case réservée pour la désignation d'États qui sont devenus parties au PCT après la publication de la présente feuille :
☐ .....

Déclaration concernant les désignations de précaution : outre les désignations faites ci-dessus, le déposant fait aussi conformément à la règle 4.9.b) toutes les désignations qui seraient autorisées en vertu du PCT, à l'exception de toute désignation indiquée dans le cadre supplémentaire comme étant exclue de la portée de cette déclaration. Le déposant déclare que ces désignations additionnelles sont faites sous réserve de confirmation et que toute désignation qui n'est pas confirmée avant l'expiration d'un délai de 15 mois à compter de la date de priorité doit être considérée comme retirée par le déposant à l'expiration de ce délai. *(La confirmation (y compris les taxes) doit parvenir à l'office récepteur dans le délai de 15 mois.)*

Cadre n° VI REVENDICATION DE PRIORITÉ ☐ D'autres revendications de priorité sont indiquées dans le cadre supplémentaire.

Date de dépôt de la demande antérieure *(jour/mois/année)*	Numéro de la demande antérieure	Lorsque la demande antérieure est une : demande nationale : pays	demande régionale :* office régional	demande internationale : office récepteur
(1) 17/09/99	9911677	FRANCE		
(2)				
(3)				

☒ L'office récepteur est prié de préparer et de transmettre au Bureau international une copie certifiée conforme de la ou des demandes antérieures *(seulement si la demande antérieure a été déposée auprès de l'office qui, aux fins de la présente demande internationale, est l'office récepteur)* indiquées ci-dessus au(x) point(s) : _____

* *Si la demande antérieure est une demande ARIPO, il est obligatoire d'indiquer dans le cadre supplémentaire au moins un pays partie à la Convention de Paris pour la protection de la propriété industrielle pour lequel cette demande antérieure a été déposée (règle 4.10.b)ii)). Voir le cadre supplémentaire.*

Cadre n° VII ADMINISTRATION CHARGÉE DE LA RECHERCHE INTERNATIONALE

Choix de l'administration chargée de la recherche internationale (ISA) *(si plusieurs administrations chargées de la recherche internationale sont compétentes pour procéder à la recherche internationale, indiquer l'administration choisie; le code à deux lettres peut être utilisé)* : ISA/

Demande d'utilisation des résultats d'une recherche antérieure; mention de cette recherche *(si une recherche antérieure a été effectuée par l'administration chargée de la recherche internationale ou demandée à cette dernière)* :

Date *(jour/mois/année)*	Numéro	Pays *(ou office régional)*
17/09/99	9911677	FRANCE

Cadre n° VIII BORDEREAU; LANGUE DE DÉPÔT

La présente demande internationale contient le **nombre de feuilles** suivant :

requête	:	4
description (sauf partie réservée au listage des séquences)	:	20
revendications	:	2
abrégé	:	1
dessins	:	15
partie de la description réservée au listage des séquences	:	
Nombre total de feuilles	:	42

Le ou les éléments cochés ci-après sont **joints** à la présente demande internationale :

1. ☒ feuille de calcul des taxes
2. ☐ pouvoir distinct signé
3. ☐ copie du pouvoir général; numéro de référence, le cas échéant :
4. ☐ explication de l'absence d'une signature
5. ☐ document(s) de priorité indiqué(s) dans le cadre n° VI au(x) point(s) :
6. ☐ traduction de la demande internationale en (langue) :
7. ☐ indications séparées concernant des micro-organismes ou autre matériel biologique déposés
8. ☐ listage des séquences de nucléotides ou d'acides aminés sous forme déchiffrable par ordinateur Copie du rapport de recherche
9. ☒ autres éléments *(préciser)* : de la D.F. 9911677

Figure des dessins qui doit accompagner l'abrégé : Néant

Langue de dépôt de la demande internationale : Français

Cadre n° IX SIGNATURE DU DÉPOSANT OU DU MANDATAIRE

À côté de chaque signature, indiquer le nom du signataire et, si cela n'apparaît pas clairement à la lecture de la requête, à quel titre l'intéressé signe.

LE GUEN Gérard
JACOBSON Claude
OBOLENSKY Michel
MONCHENY Michel
CABINET LAVOIX
2, Place d'Estienne d'Orves
75441 PARIS CEDEX 09 FRANCE

Paris, le 15 septembre 2000

L'Un des Mandataires

JACOBSON Claude

Réservé à l'office récepteur

1. Date effective de réception des pièces supposées constituer la demande internationale :
2. Dessins : ☐ reçus : ☐ non reçus :
3. Date effective de réception, rectifiée en raison de la réception ultérieure, mais dans les délais, de documents ou de dessins complétant ce qui est supposé constituer la demande internationale :
4. Date de réception, dans les délais, des corrections demandées selon l'article 11.2) du PCT :
5. Administration chargée de la recherche internationale (si plusieurs sont compétentes) : ISA/
6. ☐ Transmission de la copie de recherche différée jusqu'au paiement de la taxe de recherche.

Réservé au Bureau international

Date de réception de l'exemplaire original par le Bureau international :

TRAITE DE COOPERATION EN MATIERE DE BREVETS

Expéditeur: le BUREAU INTERNATIONAL

PCT

NOTIFICATION D'ELECTION

(règle 61.2 du PCT)

Destinataire:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

en sa qualité d'office élu

Date d'expédition (jour/mois/année) 15 août 2001 (15.08.01)	
Demande internationale no PCT/FR00/02577	**Référence du dossier du déposant ou du mandataire** BET 00/0866
Date du dépôt international (jour/mois/année) 15 septembre 2000 (15.09.00)	**Date de priorité** (jour/mois/année) 17 septembre 1999 (17.09.99)
Déposant SEPULCHRE, Anne-Marie etc	

1. L'office désigné est avisé de son élection qui a été faite:

[X] dans la demande d'examen préliminaire international présentée à l'administration chargée de l'examen préliminaire international le:

14 mars 2001 (14.03.01)

[ ] dans une déclaration visant une élection ultérieure déposée auprès du Bureau international le:

2. L'élection [X] a été faite

[ ] n'a pas été faite

avant l'expiration d'un délai de 19 mois à compter de la date de priorité ou, lorsque la règle 32 s'applique, dans le délai visé à la règle 32.2b).

Bureau international de l'OMPI
34, chemin d s Colombettes
1211 Genèv 20, Suisse

no de télécopieur: (41-22) 740.14.35

Fonctionnaire autorisé

Antonia Muller

no de téléphone: (41-22) 338.83.38

UTILISATION DE SOUCHES STREPTOCOCCUS THERMOPHILUS INCAPABLES D'HYDROLYSER L'UREE DANS DES PRODUITS LAITIERS

La présente invention concerne la maîtrise de la cinétique d'acidification du lait lors de la fabrication de fromages ou de laits fermentés tels que des yaourts, par la mise en œuvre de bactéries *Streptococcus thermophilus* au moins partiellement, de préférence totalement, incapables d'hydrolyser l'urée.

Streptococcus thermophilus est une bactérie lactique thermophile utilisée comme ferment lactique dans l'industrie laitière. Employée tout d'abord pour la fabrication de laits fermentés tels que le yaourt, elle est maintenant de plus en plus mise en œuvre dans la production de fromages.

Cette bactérie transforme le lactose en acide lactique, et présente par là une activité acidifiante. Dans le cas des fromages notamment, cette acidification non seulement favorise l'action de la présure et la synérèse du caillé mais encore inhibe la croissance de nombreuses bactéries indésirables, dont certaines sont des bactéries pathogènes, et permet même plus ou moins rapidement leur élimination.

L'activité acidifiante de cette bactérie est cependant doublée d'une activité d'hydrolyse de l'urée, activité qui affecte la cinétique d'acidification. Tinson et al (1982a) ont montré que la réaction d'hydrolyse de l'urée, donnant du dioxyde de carbone et de l'ammoniaque, induisait une diminution temporaire de la vitesse d'acidification, mesurée par une sonde de pH. Les auteurs de cet article en concluent qu'on ne peut pas utiliser les changements de pH pour mesurer la production d'acide lactique dans des cultures de *S. thermophilus*, car les résultats qu'on obtiendrait seraient erronés en raison de la production d'ammoniaque. Par ailleurs Spinnler et Corrieu en 1989 ont observé que l'ajout d'urée conduisait à une baisse de la vitesse d'acidification.

A l'échelle industrielle, l'hydrolyse de l'urée par *Streptococcus thermophilus* pose un certain nombre de problèmes.

En effet, dans les fabrications fromagères par exemple, les opérations technologiques (découpage du caillé, brassage etc.) doivent avoir lieu à des valeurs de pH données, mais en pratique, ces opérations sont généralement réalisées à des temps déterminés. De ce fait, les variations d'activité acidifiante dues à l'hydrolyse de l'urée entraînent des défauts et des variabilités importantes dans les fromages (texture, taux d'humidité, affinage). Martin et al (1997) ont ainsi observé que les variations des teneurs en urée, provoquaient des modifications dans les cinétiques d'acidification et dans la texture des fromages de type reblochon, confirmant les résultats obtenus par Spinnler et Corrieu (1989).

En outre, la production d'ammoniaque augmente le temps nécessaire pour atteindre un pH donné. Ceci se traduit par une immobilisation plus importante du matériel ainsi que par une augmentation du risque de contamination par des micro-organismes indésirables.

Par ailleurs, il est souhaitable que le lactosérum de fromagerie ne contienne pas une quantité excessive d'ammoniaque, car ce lactosérum est souvent utilisé en alimentation animale.

Ce phénomène est difficilement maîtrisable, notamment parce que la teneur du lait en urée est variable (généralement de 2 à 8 mM) et qu'elle dépend en particulier de l'alimentation du bétail. Pour pallier ce problème, Martin et al (1997) ont proposé de mesurer les teneurs en urée du lait et d'adapter ensuite les paramètres de fabrication. Cependant la mise en œuvre d'un tel système de dosage de l'urée serait très contraignante, et ne résoudrait de toute façon pas les inconvénients dus à un ralentissement de la vitesse d'acidification en présence d'urée (durée d'immobilisation plus importante du matériel, augmentation des risques de contamination etc.) et à une teneur élevée du lactosérum en ammoniaque.

Les auteurs de la présente invention ont mis en évidence que l'utilisation de souches *Streptococcus thermophilus* n'hydrolysant pas, ou pas totalement, l'urée, comme ferments lactiques dans la production de produits

laitiers, permettait de résoudre les problèmes précités. Ces souches sont désignées "souches ur(-)", dans la suite de cette demande.

Jusqu'à présent, les seules souches *Streptococcus thermophilus* ur(-) décrites sont la souche CNRZ 407 (Juilliard et al, 1988) et la souche mutante isolée par Tinson et al (1982b). Cependant, les informations connues relatives à ces deux souches ne permettent pas de se rendre compte de l'intérêt technologique des souches ur(-).

La présente invention a donc pour objet l'utilisation d'au moins une souche *Streptococcus thermophilus* au moins partiellement, de préférence totalement, incapable d'hydrolyser l'urée, lors de la fabrication de fromages ou de produits laitiers fermentés tels que des yaourts, pour obtenir une cinétique d'acidification substantiellement indépendante de la teneur du lait en ses composants.

Dans le cadre de la présente invention, on entend par "la cinétique d'acidification" la variation du pH du milieu de fermentation en fonction du temps.

Par « teneur du lait en ses composants», on entend en particulier les teneurs en urée des laits, qui diffèrent d'un lait à l'autre, selon l'origine de l'animal ou son alimentation. On entend également les teneurs en d'autres composants du lait qui sont impliqués dans le métabolisme de l'urée. Parmi ces composants, on peut citer par exemple le nickel ou le cobalt. Ces composants peuvent être présents naturellement dans la matière première utilisée (le lait) ou avoir été ajoutés.

La présente invention a également pour objet un procédé pour obtenir, lors de la fabrication de fromages ou de produits laitiers fermentés tels que des yaourts, une cinétique d'acidification substantiellement indépendante de la teneur du lait en ses composants, dans lequel on incorpore au lait au moins une souche *Streptococcus thermophilus*, au moins partiellement, de préférence totalement, incapable d'hydrolyser l'urée.

Les souches *Streptococcus thermophilus* ur(-) mises en œuvre conformément à la présente invention peuvent être obtenues par un traitement mutagène ou par mutation spontanée, ou encore être isolées dans la nature.

Les souches 298-K et 298-10, qui sont respectivement un mutant spontané et un mutant obtenu après traitement mutagène, ont été déposées à la CNCM le 14 septembre 1999 sous les numéros I-2311 et I-2312, respectivement.

Toute souche ur(-) criblée selon le protocole de Tinson et al (1982b), ou de préférence selon le protocole décrit dans l'exemple I, peut également être utilisée.

Les souches *Streptococcus thermophilus* ur(-) peuvent être utilisées seules ou en mélange avec d'autres microorganismes tels que des lactocoques, des lactobacilles, ou tout autre microorganisme utilisable dans l'industrie laitière.

Les auteurs de la présente invention ont montré que l'intérêt des souches *Streptococcus thermophilus* ur(-) est multiple. En effet, ils ont mis en évidence que les mutants ur(-) permettent non seulement de maîtriser les variations des cinétiques d'acidification, mais qu'ils sont en outre stables et présentent une bonne croissance dans le lait.

Par ailleurs, les souches ur(-) permettent d'obtenir des cinétiques d'acidification du lait régulières, qui ne présentent pas de ralentissement temporaire, fonction de la concentration en urée, contrairement aux cinétiques observées avec les souches ur(+).

Les souches ur(-) ne produisent pas d'ammoniaque lors de leur croissance dans du lait, ce qui est avantageux dans l'optique d'une utilisation du lactosérum dans l'alimentation animale.

Enfin, les souches sélectionnées pour leur phénotype ur(-) présentent de manière surprenante des caractères acidifiants variables, par rapport aux cinétiques d'acidification observées avec les souches parentales.

Par "cinétique d'acidification variable", on entend une cinétique d'acidification par exemple plus rapide ou plus lente par rapport aux cinétiques d'acidification observées avec les souches parentales. On peut aussi parler

"d'hétérogénéité" entre les cinétiques d'acidification des différents mutant ur(-) vis-à-vis des souches parentales.

L'invention a donc également pour objet un procédé de sélection de souches *Streptococcus thermophilus* utiles lors de la fabrication de fromages ou de produits laitiers fermentés, dans lequel des souches *Streptococcus thermophilus* mutantes, au moins partiellement, de préférence totalement incapables d'hydrolyser l'urée, permettant l'obtention d'une cinétique d'acidification substantiellement indépendante de la teneur du lait en ses composants, sont sélectionnées pour leur capacité à acidifier un lait selon des cinétiques d'acidification variables par rapport aux cinétiques d'acidification des souches parentales.

De manière générale, le choix des propriétés acidifiantes des souches ur(-) peut être effectué en fonction de la technologie de fabrication fromagère ou de laits fermentés, pour laquelle ces souches sont mises en oeuvre.

Ainsi, certaines souches ur(-) se caractérisent plus particulièrement par une absence du phénomène de post-acidification.

Pour d'autres souches, le temps nécessaire pour atteindre un pH donné s'avère plus court que pour les souches ur(+) parentales. Ainsi, cette propriété permet d'ensemencer le lait avec une souche mutante ur(-) à un taux inférieur au taux généralement utilisé pour la souche ur(+) parentale. Ce taux peut être inférieur d'environ 25 %, voire d'environ 50 % par rapport au taux qui serait utilisé pour la souche parentale.

La présente invention a donc pour objet un procédé selon l'invention, dans lequel on incorpore au lait au moins une souche *Streptococcus thermophilus* mutante au moins partiellement, de préférence totalement incapable d'hydrolyser l'urée, à un taux d'ensemencement inférieur au taux d'ensemencement utilisé pour la souche *Streptococcus thermophilus* parentale capable d'hydrolyser l'urée.

Les figures et exemples ci-après illustrent l'invention sans en limiter la portée.

LEGENDE DES FIGURES :

La figure 1 représente des courbes d'acidification de lait écrémé reconstitué, obtenues avec la souche RD298 ur(+) ainsi qu'avec les mutants ur(-) spontanés (figure 1A) ou obtenus après un traitement au NTG (figure 1B).

La figure 2 représente les courbes d'acidification de lait écrémé reconstitué, obtenues avec la souche ST888 ainsi qu'avec les mutants ur(-) spontanés (figure 2A) ou obtenus après un traitement au NTG (figure 2B).

La figure 3 représente les courbes d'acidification de lait écrémé UHT obtenues avec la souche RD298 ainsi qu'avec les mutants ur(-) spontanés (figure 3A) ou obtenus après un traitement au NTG (figure 3B).

La figure 4 représente des courbes d'acidification de lait écrémé UHT, obtenues avec la souche ST888 ainsi qu'avec les mutants ur(-) spontanés (figure 4A) ou obtenus après un traitement au NTG (figure 4B).

La figure 5 représente les courbes d'acidification obtenues avec la souche RD298 (figure 5A) et les mutants ur(-) RD 298-K (figure 5B) et RD298-10 (figure 5C), sur du lait écrémé UHT supplémenté avec différentes quantités d'urée.

La figure 6 représente les courbes d'acidification obtenues avec la souche RD298 (figure 6A) et les mutants ur(-) RD 298-K (figure 6B) et RD298-10 (figure 6C), sur du lait écrémé UHT supplémenté ou non avec du nickel (10 µg/l de $NiSO_4.7\ H_2O$).

La figure 7 représente les courbes d'acidification obtenues avec la souche RD672 et des mutants ur(-) issus de cette souche, sur du lait écrémé reconstitué.

EXEMPLES :

Exemple 1 :

Méthode de criblage des bactéries ur(-) sur boîte de Pétri

Un milieu gélosé dont la composition est indiquée dans le tableau 1 est préparé et coulé dans des boîtes de Pétri d'un diamètre égal à 9 cm.

Tableau 1 : Composition du milieu de criblage.

Tryptone[a]	2,5 g
Peptone pepsique de viande[a]	2,5 g
Peptone papaïnique de soja[a]	5 g
Extrait autolytique de levure[b]	2,5 g
Extrait de viande[a]	5 g
Sucre (glucose, lactose ou saccharose)	5 g
Glycérophosphate de sodium.$6H_2O$	19 g
Sulfate de magnésium	0,25 g
Acide ascorbique	0,5 g
Agar	15 g
Eau distillée	1 l

[a] : Société Biokar

[b] : Société Fischer Scientific

Le cas échéant, on peut ajouter à ce milieu un cofacteur de l'uréase. Ajuster le pH à 7,0 et autoclaver pendant 15 minutes à 115°C.

Les cellules de *St. thermophilus* à analyser sont ensemencées sur ce milieu de manière à obtenir environ 100 colonies par boîte de Pétri. Les cultures ont lieu en anaérobiose à une température de 35-45°C, de préférence 37-42°C.

Après deux jours de culture, on verse sur chaque boîte de pétri environ 20 ml d'une solution gélosée préparée de la façon suivante : dissoudre par chauffage 15 g d'agar dans 1 litre d'une solution de tampon phosphate de potassium à 50 mM (pH 6) supplémentée avec 100 mg/l de bleu de bromothymol, refroidir la solution à 50°C, ajouter 10 g d'urée et acidifier le milieu avec de l'acide chlorhydrique jusqu'à l'obtention d'une couleur jaune-orange.

Après solidification de la gélose, les boîtes de Pétri sont incubées 1 h à 37°C. Les clones ur(+) forment des halos de couleur bleue en raison de la production d'ammoniaque, alors que les clones ur(-) forment des colonies jaunes. Lorsque les mutants ur(-) sont recherchés, les clones ne formant pas de halo bleu sont récupérés et testés à nouveau sur le même milieu de criblage afin de confirmer le caractère ur(-). Il convient également de vérifier que ces mutants ne consomment pas l'urée (ou ne le consomment qu'en partie) lorsqu'ils sont cultivés dans du lait.

Exemple 2 :

Sélection de mutants du métabolisme de l'urée

Des mutants ne consommant pas l'urée, ou le consommant faiblement, ont été recherchés à partir des souches de *St. thermophilus* RD298, RD 672 et ST888. Deux approches ont été utilisées. Dans la première approche, les mutants ont été recherchés après un traitement avec un agent mutagène, alors que dans la seconde approche, des mutants spontanés ont été recherchés.

a) Sélection à l'aide d'un agent mutagène

Le traitement mutagène est réalisé comme décrit ci-dessous.

Les souches sont cultivées à 42°C dans 5 ml de bouillon M17 (Terzaghi et Sandine, 1975). La culture est arrêtée en fin de phase exponentielle, et les cellules sont récupérées par centrifugation puis lavées avec du tampon phosphate 100 mM (pH 7). Les cellules sont ensuite récupérées dans 1 ml de tampon contenant une teneur variable en N-méthyl-N'-nitro-N-nitrosoguanidine (NTG) et incubées pendant 1 heure à 42°C. Les

cellules sont ensuite lavées deux fois avec 5 ml de tampon et ensemencées sur le milieu de criblage de manière à obtenir environ 100 colonies par boîte de Pétri. Le criblage est réalisé comme décrit précédemment (exemple 1). Le tableau 2 décrit les résultats obtenus lors de 3 mutagenèses.

Tableau 2 : Sélection de mutants ur (-) après un traitement avec un agent mutagène (NTG).

Souche de *St. thermophilus*	Concentration en NTG utilisée (µg/ml)	Viabilité (% des cellules ayant survécu au NTG)	Nbre de colonies criblées	Nbre de clones ur(-) obtenus	proportion des clones ur(-) (%)
ST888	20	10	980	11	1,1
ST888	5	48	1000	5	0,5
RD672	50	41	10600	41	0,4
RD298	50	16	3200	15	0,5

b) Sélection de mutants spontanés

Dans une population de microorganismes, il existe souvent des mutants spontanés pour un gène ou un caractère donné. Ce type de mutant est très intéressant, car le fait qu'aucun agent mutagène n'ait été utilisé supprime le risque d'induction de mutations non recherchées (autres que pour le caractère étudié), qui pourraient altérer les aptitudes technologiques des souches. Cependant, la fréquence de mutants spontanés au sein d'une population pour un caractère donné est généralement très faible, de l'ordre de 1 sur 1 million (variable en fonction des souches et des caractères). De ce fait, la sélection de mutants spontanés nécessite généralement, soit la mise au point d'une méthode permettant de cribler un nombre très élevé de clones, soit de définir une procédure d'enrichissement des mutants. Aucune procédure d'enrichissement de mutants ur(-) n'a été *a priori* décrite. De plus, étant donné que la procédure de criblage sur boîte de Pétri ne permet pas d'analyser plus de 100 colonies de *St. thermophilus* par boîte, on pouvait s'attendre à ce que la

sélection de mutants spontanés soit irréalisable, puisqu'il aurait fallu cribler plusieurs milliers, voire dizaines de milliers, de boîtes de pétri, pour avoir des chances d'isoler un mutant spontané. Or, les auteurs de la présente invention se sont aperçus que dans les cultures de *St. thermophilus*, la proportion de mutants ur(-) spontanés était élevée (environ 1 sur 2500 pour ST888, 1 sur 4000 pour RD672 et 1 sur 1200 pour RD298), et qu'il est donc possible d'isoler facilement ce type de mutant (tableau 3).

Tableau 3 : Sélection de mutants ur(-) spontanés. Le protocole utilisé est le même que celui décrit dans le paragraphe a) "sélection à l'aide d'un agent mutagène", sauf que l'agent mutagène est omis.

Souche de *St. thermophilus*	Nbre de colonies criblées	Nbre de clones ur(-) obtenus	proportion des clones ur(-) (%)
ST888	16000	6	0,04
RD298	7400	6	0,08
RD672	24000	6	0,03

47 des 90 mutants obtenus ont été étudiés. Les résultats concernant la stabilité, la caractérisation enzymatique, ainsi que le comportement acidifiant de ces mutants sont décrits ci-dessous.

Exemple 3 :

Propriétés des mutants ur(-)

a) Stabilité des mutants

Pour pouvoir être utilisables dans un contexte industriel, les mutants ur(-) doivent être stables. Or il n'existait aucune donnée quant à la stabilité de mutants ur(-) de *St. thermophilus*. Les auteurs de la présente invention ont étudié la stabilité de 47 mutants issus des souches ST888, RD 672 et RD298. Les souches ont été repiquées quotidiennement dans 10 ml de bouillon M17, et cela pendant 20 jours. Les cultures étaient inoculées à 1 % et

incubées à 42°C. L'ensemble des 20 repiquages représente environ 130 générations. Après le 20ème repiquage, les souches ont été ensemencées dans du lait et l'on a déterminé si elles consommaient ou non l'urée (cultures de 15 h à 42°C). Les résultats sont présentés dans le tableau 4. On constate que les mutants ur(-), qu'ils soient obtenus par un traitement mutagène ou qu'il s'agisse de mutants spontanés, sont très stables. En effet, seules deux réversions ont été détectées pour les 47 mutants testés.

Tableau 4 : Etude de la stabilité des mutants ur(-). La consommation d'urée a été testée lors de cultures sur du lait, après 20 repiquages successifs dans du bouillon M17.

Souche de *St. thermophilus*	Mutation	Nbre de mutants ur(-) testés	Nbre de mutants consommant l'urée après 20 repiquages
ST888	NTG	6	1
ST888	Spontanée	6	0
RD298	NTG	5	0
RD298	Spontanée	6	0
RD672	NTG	19	0
RD672	Spontanée	5	1
Total	/	47	2

b) Caractérisation enzymatique des mutants

Les souches étudiées ont été cultivées pendant 24 h, en anaérobiose et à 37 °C, dans un bouillon liquide dont la composition est indiquée dans le tableau 5. Les cellules ont été récupérées par centrifugation, lavées dans du tampon (HEPES 50 mM – EDTA 1 mM, pH 7,5), puis récupérées dans un volume de tampon représentant 2% du volume de la culture. L'activité uréasique a ensuite été mesurée sur des extraits acellulaires (traitement des cellules dans un broyeur à billes et récupération du surnageant de centrifugation pendant 5 min à 20000 g).

Tableau 5 : Composition du bouillon utilisé pour la préparation des extraits.

Tryptone[a]	10 g
Extrait autolytique de levure[b]	5 g
Glycérophosphate de sodium, $6H_2O$	19 g
Acide ascorbique	500 mg
Sulfate de magnésium	250 mg
Sulfate de nickel.$7H_2O$	10 mg
Glucose	10 g
Eau distillée	1 l

[a]: Société Biokar

[b]: Société Fischer Scientific

Ajuster le pH à 7,0 et autoclaver pendant 15 minutes à 115°C.

Les mesures d'activité uréasique ont été réalisées à 37°C, dans du tampon HEPES 50 mM – EDTA 1 mM (pH 7,5). La réaction est déclenchée par l'ajout de 25 mM d'urée, et l'on dose l'ammoniaque produit en 20 minutes, en utilisant le réactif de Nessler. Les résultats sont exprimés en unités (U) d'activité uréase (une unité correspond à une micromole d'ammoniaque produite par minute) par milligramme de protéine.

Le tableau 6 présente les valeurs d'activité obtenues. Les mutants ur(-) ne présentaient pas d'activité uréasique détectable, à l'exception des mutants 298-3.17 et 888-1.5. Ces derniers correspondent à des mutants ayant un phénotype ur(+) en présence de nickel et ur(-) en absence de ce composé. Or, le milieu de culture utilisé pour la préparation des extraits acellulaires contenait du sulfate de nickel. Dans ces deux souches, la mutation porte probablement sur le système de transport du nickel ou sur le système permettant son incorporation dans le site actif de l'uréase.

Ces souches de St. *thermophilus* pourraient également présenter un phénotype ur(-) du fait d'une incapacité à transporter l'urée. De telles

souches posséderaient donc toujours une activité uréasique mesurable dans des extraits acellulaires.

Tableau 6 : Mesure de l'activité uréasique d'extraits acellulaires obtenus à partir des souches parentales ainsi que des mutants ur(-).

Souche parentale Mutant	Activité uréasique (U/mg)	Souche parentale Mutant	Activité uréasique (U/mg)	Souche parentale Mutant	Activité uréasique (U/mg)
RD298	0,94	RD672	1,08	ST888	0,95
298-10	N.D.	672-18(0)	N.D.	888-A	N.D.
298-K	N.D.	672-47(0)	N.D.	888-B	N.D.
298-I	N.D.	672-54(0)	N.D.	888-C	N.D.
298-J	N.D.	672-19(0)	N.D.	888-D	N.D.
298-L	N.D.	672-31(0)	N.D.	888-1	N.D.
298-M	N.D.	672-59(50)	N.D.	888-2	N.D.
298-N	N.D.	672-62(50)	N.D.	888-2,6	N.D.
298-3,9	N.D.	672-61(50)	N.D.	888-2,11	N.D.
298-3,3	N.D.	672-33(50)	N.D.	888-2,9	N.D.
298-3,16	N.D.	672-55(50)	N.D.	888-1,13	N.D.
298-3,17	0,58	672-53(50)	N.D.	888-1,8	N.D.
		672-70(50)	N.D.	888-1,5	0,42
		672-20(50)	N.D.		
		672-50(50)	N.D.		
		672-34(50)	N.D.		
		672-22(50)	N.D.		
		672-24(50)	N.D.		
		672-10(50)	N.D.		
		672-36(50)	N.D.		
		672-60(50)	N.D.		
		672-21(50)	N.D.		
		672-27(50)	N.D.		
		672-26(50)	N.D.		
		672-41(50)	N.D.		

N.D. Non Détecté

c) Comportement acidifiant des mutants

Afin de démontrer l'intérêt technologique des souches ur(-), les auteurs de l'invention ont comparé leurs caractéristiques acidifiantes avec celles des souches parentales correspondantes.

Il a été observé les résultats suivants :

- contrairement aux souches parentales, les mutants ur(-) ne présentent pas un ralentissement temporaire de la vitesse d'acidification dû à l'hydrolyse de l'urée, leurs courbes d'acidification sont donc plus régulières ;

- Les cinétiques d'acidification du lait par les mutants ur(-) sont peu ou pas affectées par les teneurs en urée, en nickel et en cobalt ;

- par ailleurs, on observe une forte variabilité des activités acidifiantes entre les mutants ur(-), par rapport aux activités acidifiantes des souches parentales.

Le détail des résultats obtenus est présenté ci-dessous. Les cultures ont été ensemencées à 1% avec une préculture réalisée sur du lait écrémé reconstitué stérilisé, puis cultivées à 37°C.

- Cultures dans du lait écrémé reconstitué :

Le lait a été reconstitué à 100 g/l et pasteurisé pendant 10 minutes à 90°C.

Après environ 2 heures de culture, on observe une remontée du pH dans la culture de la souche RD298 (figure 1). Les 6 mutants spontanés présentent une courbe d'acidification plus régulière, sans remontée de pH ni ralentissement temporaire de la vitesse d'acidification. A certains moments de la culture, le décalage d'acidification par rapport à la souche parentale atteint près de 4 heures. Ceci permet donc d'atteindre plus rapidement une valeur de pH donnée. L'intérêt de cette observation est majeure : si l'on veut atteindre un pH donné sans diminuer la durée d'incubation, on peut utiliser une souche ur(-) en diminuant la quantité d'ensemencement par rapport à la quantité utilisée avec une souche ur(+). Certains des mutants obtenus après un traitement au NTG ont un comportement similaire aux mutants spontanés, d'autres acidifient le milieu plus lentement (298-3.3) ou plus rapidement (298-10).

A l'exception du mutant 888-1, les mutants spontanés ur(-) de ST888 présentent la même courbe d'acidification. Comme pour RD298, on observe une acidification plus régulière et plus rapide avec les mutants (figure 2).

- Cultures dans du lait écrémé stérilisé UHT (Lactel®) :

Comme pour les cultures réalisées dans du lait reconstitué, on observe un arrêt temporaire de la baisse du pH avec la souche RD298, ce phénomène étant absent dans les cultures des mutants ur(-) spontanés (figure 3).

Les mutants ur(-) isolés à partir de ST888, qu'ils soient spontanés ou obtenus par traitement au NTG, ont une courbe d'acidification plus régulière que celle de la souche parentale (figure 4).

- Effet de variations de la composition du lait sur les courbes d'acidification :

La souche RD298, ainsi que les mutants ur(-) 298-K et 298-10, ont été cultivés sur du lait écrémé stérilisé UHT supplémenté ou non avec différentes quantités d'urée. La concentration initiale du lait en urée était égale à 3 mM et les teneurs en urée des différentes cultures étaient comprises dans les zones de variation que l'on observe habituellement avec le lait de vache. On constate que, contrairement aux mutants ur(-), les courbes d'acidification obtenues avec la souche parentale sont très dépendantes de la teneur du lait en urée (figure 5).

Les auteurs de la présente invention ont également observé que les courbes d'acidification obtenues avec la souche parentale sont dépendantes de la teneur du lait un nickel et en cobalt, ce qui n'est pas le cas pour les mutants ur(-) (figure 6).

- Production d'ammoniaque :

Dans toutes les cultures décrites précédemment, on a observé que les souches RD298 et ST888 produisaient de l'ammoniaque et hydrolysaient la totalité de l'urée contenue dans le lait. Aucune production d'ammoniaque n'a été observée avec les mutants. Ceci indique que l'urée est le principal substrat utilisé par *St. thermophilus* pour produire de l'ammoniaque.

Ainsi, l'utilisation de souches ur(-) permet d'éviter toute production d'ammoniaque due à *St. thermophilus* lors des fabrications fromagères. Par suite, les teneurs en ammoniaque des lactosérums de fromagerie peuvent être limitées.

- *Variabilité des activités acidifiantes :*

Les auteurs de la présente invention ont observé de manière intéressante que les courbes d'acidification dans du lait écrémé reconstitué, obtenues avec plusieurs souches mutantes ur(-) présentaient d'importantes variations par rapport à la courbe obtenue avec leur souche parentale.

La figure 7 montre ainsi les courbes d'acidification de lait écrémé reconstitué, obtenues avec la souche RD 672, ainsi qu'avec des mutants ur(-) issus de cette souche.

La souche RD672 est peu acidifiante (technologie de type pâte molle solubilisée). Le mutant 672-47(0) est nettement plus acidifiant que la souche parentale, tandis que le mutant 672-36(50) présente une cinétique d'acidification assez proche. Le mutant 672-70(0) est nettement moins acidifiant que la souche parentale et le mutant 672-24(50) est un peu moins acidifiant que la souche parentale.

Exemple 4 :

Fabrication de fromages de type "pâte molle solubilisée" mettant en œuvre soit la souche industrielle ur(+) RD298 soit la souche mutante ur(-) 298-10 (mutante de RD298)

a) Généralités.

Sous le nom générique de fromage, se trouve un très grand nombre de produits, ayant une technologie, une flore et des propriétés organoleptiques très diverses.

Sur le plan technologique, le fromage résulte dans un premier temps de la coagulation du lait obtenue par l'emprésurage, qui sera suivie de l'égouttage du coagulum ainsi obtenu (opérations mécaniques telles que le découpage, le brassage et le retournement).

Au cours de la fabrication, le développement des ferments ajoutés va provoquer un abaissement du pH du coagulum. La cinétique d'acidification (évolution du pH en fonction du temps) et la cinétique d'égouttage conditionnent la composition finale du caillé et donc les caractéristiques intrinsèques des fromages. C'est pourquoi, pour une technologie donnée, la maîtrise des cinétiques d'acidification et d'égouttage est essentielle.

b) Spécificités de la technologie "pâte molle solubilisée" mise en œuvre.

La fabrication des fromages de type "pâte molle solubilisée" correspond à la mise en œuvre d'une technologie à dominance enzymatique (rôle important de la présure) avec des profils de température de fabrication spécifiques, tel que celui décrit dans le tableau 7.

La conduite de l'égouttage se caractérise par :

- Une acidification importante en début de procédé qui conditionne le niveau d'égouttage. L'acidification est assurée par *Streptococcus thermophilus* ; les pH cibles à atteindre aux différents stades de fabrication sont résumés dans le tableau 7.

- Une évacuation rapide du sérum accentuée par des opérations mécaniques (découpage, brassage et moulage du coagulum).

- Des opérations facilitant l'évacuation du lactosérum (retournement).

c) Suivi des fabrications fromagères

Le tableau 7 résume les différentes étapes technologiques des fabrications réalisées et rapporte les temps technologiques qui ont été nécessaires dans chaque essai pour atteindre les pH cibles de chacune de ces étapes.

Deux laits distincts ont été mis en œuvre contenant pour l'un moins de 1mM d'urée et pour l'autre 5 mM d'urée. Les ferments utilisés étaient constitués soit de la souche industrielle RD298 connue pour sa capacité à

hydrolyser l'urée ur(+) soit de la souche 298-10, un mutant spontané de cette souche dépourvu de cette capacité d'hydrolyse de l'urée ur(-).

Les suivis d'acidification du lait contenant une quantité très faible d'urée (moins de 1mM) montrent que les deux souches mises en œuvre permettent d'atteindre les pH cibles de chaque étape dans des temps approximativement identiques. De la même façon, ces objectifs sont atteints avec la souche 298-10 ur(-) lorsque le lait de fabrication contient des quantités significatives d'urée (5 mM). Au contraire, pour respecter les pH cibles de fabrication avec la souche RD298 dans le lait contenant 5 mM d'urée, les temps technologiques ont dû être considérablement allongés.

Cette étude démontre donc l'avantage technologique certain du mutant 298-10 ur(-) par rapport à la souche mère industrielle RD298 ur(+).

Tableau 7 : Caractéristiques technologiques d'une fabrication fromagère de type "pâte molle solubilisée" et description technologique de fabrications réalisées avec les souches RD298 ur(+) ou 298-10 ur(-) utilisées comme ferment à partir de lait contenant soit 5 mM d'urée soit moins de 1mM d'urée.

Stade de fabrication	Température de fabrication (°C)	pH cible (± 0,05)	Objectifs temps technologiques (± 10 min)	Temps technologique effectif (min.)			
				Lait avec moins de 1mM d'urée		Lait contenant 5 mM d'urée	
				RD.298	298-10	RD 298	298-10
Lait	38 ± 0,5	6,48	0 ± 10	0	0	0	0
Emprésurage		6,40	70 ± 10	70	60	100	60
Moulage		6,30	120 ± 10	120	110	140	110
1er retournement	35 ± 0,5	6,20	180 ± 10	190	170	280	170
2ème retournement	26 ± 0,5	5,50	300 ± 10	310	310	450	310
3ème retournement	20 ± 0,5	5,25	540 ± 10	540	530	700	530

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- Terzaghi B.E., Sandine W.E. 1975. Improved medium for lactic streptococci and their bacteriophages. Applied Microbiology. 29:807-813.

- Tinson W., Broome M.C., Hillier A.J., Jago G.R. 1982a. Metabolism of *Streptococcus thermophilus*. 2. Production of CO2 and NH3 from urea. Australian Journal of Dairy Technology. 37:14-16.

- Tinson W., Ratcliff M.F., Hillier A.J., Jago G.R. 1982b. Metabolism of *Streptococcus thermophilus*. 3. Influence on the level of bacterial metabolites in cheddar cheese. Australian Journal of Dairy Technology. 37:17-21.

REVENDICATIONS

1. Utilisation d'au moins une souche *Streptococcus thermophilus* au moins partiellement, de préférence totalement, incapable d'hydrolyser l'urée, lors de la fabrication de fromages ou de produits laitiers fermentés tels que des yaourts, pour obtenir une cinétique d'acidification substantiellement indépendante de la teneur du lait en ses composants.

2. Utilisation selon la revendication 1, dans laquelle la cinétique d'acidification est substantiellement indépendante de la teneur en urée du lait.

3. Utilisation selon la revendication 1, dans laquelle la cinétique d'acidification du lait est substantiellement indépendante de la teneur en nickel ou en cobalt du lait.

4. Utilisation selon l'une des revendications précédentes, dans laquelle la cinétique d'acidification du lait ne présente pas de ralentissement temporaire.

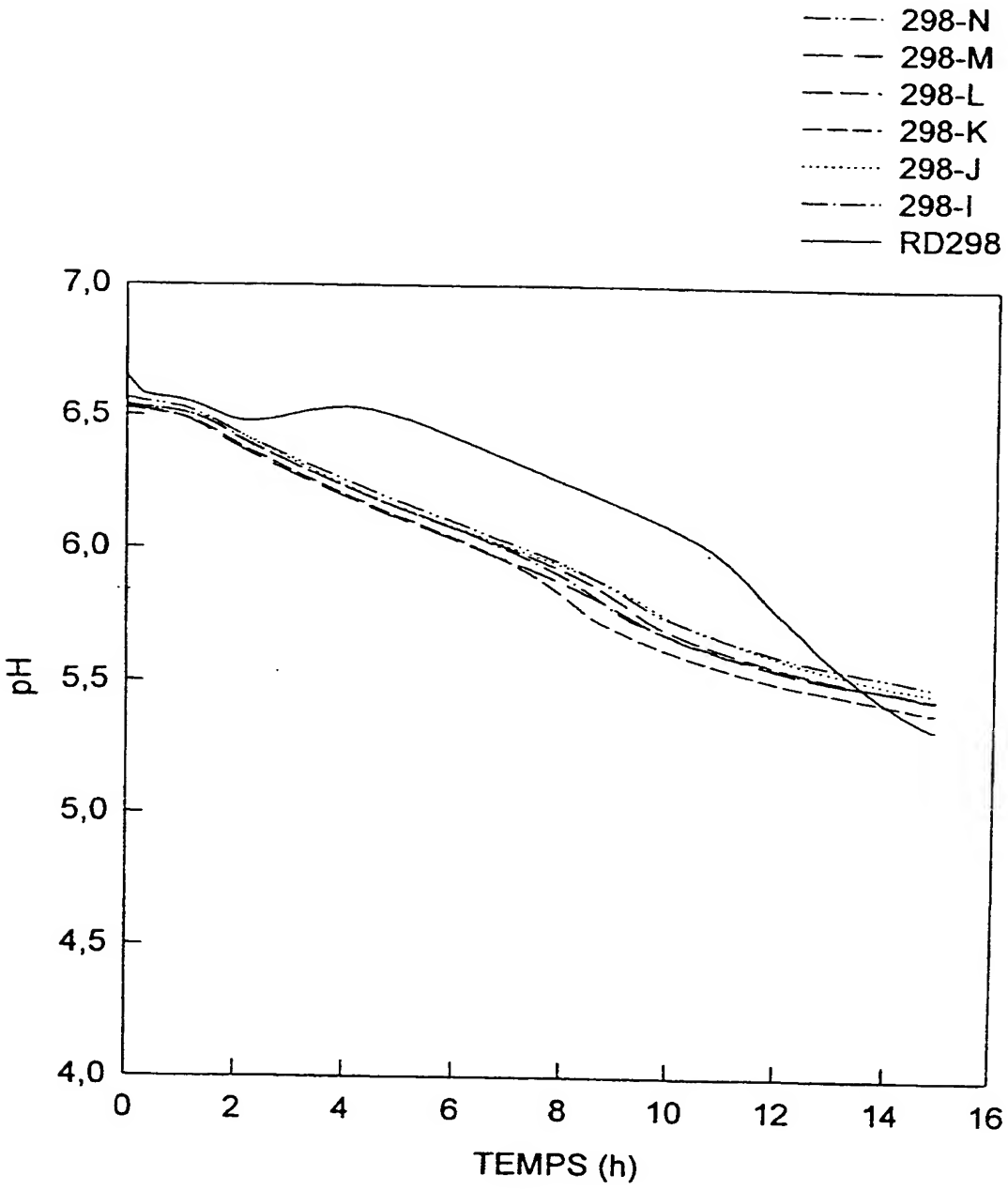
5. Utilisation selon l'une quelconque des revendications précédentes, dans lequel la souche *Streptococcus thermophilus* est la souche 298-K déposée à la CNCM sous le numéro I-2311.

6. Utilisation selon l'une quelconque des revendications 1 à 4, dans laquelle la souche *Streptococcus thermophilus* est la souche 298-10 déposée à la CNCM sous le numéro I-2312.

7. Procédé pour obtenir, lors de la fabrication de fromages ou de produits laitiers fermentés tels que des yaourts, une cinétique d'acidification substantiellement indépendante de la teneur du lait en ses composants, dans lequel on incorpore au lait au moins une souche *Streptococcus thermophilus* au moins partiellement, de préférence totalement, incapable d'hydrolyser l'urée.

8. Procédé selon la revendication 7, dans lequel on incorpore au lait au moins une souche *Streptococcus thermophilus* mutante au moins partiellement, de préférence totalement, incapable d'hydrolyser l'urée, à un taux d'ensemencement inférieur au taux d'ensemencement utilisé pour la souche *Streptococcus thermophilus* parentale capable d'hydrolyser l'urée.

9. Procédé de sélection de souches *Streptococcus thermophilus* utiles lors de la fabrication de fromages ou de produits laitiers fermentés, dans lequel des souches *Streptococcus thermophilus* mutantes, au moins partiellement, de préférence totalement incapables d'hydrolyser l'urée, permettant l'obtention d'une cinétique d'acidification substantiellement indépendante de la teneur du lait en ses composants, sont sélectionnées pour leur capacité à acidifier un lait selon des cinétiques d'acidification variables par rapport aux cinétiques d'acidification des souches parentales.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298-M
298-L
298-K
298-J
298-I
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6,0
5,5
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4,0
pH
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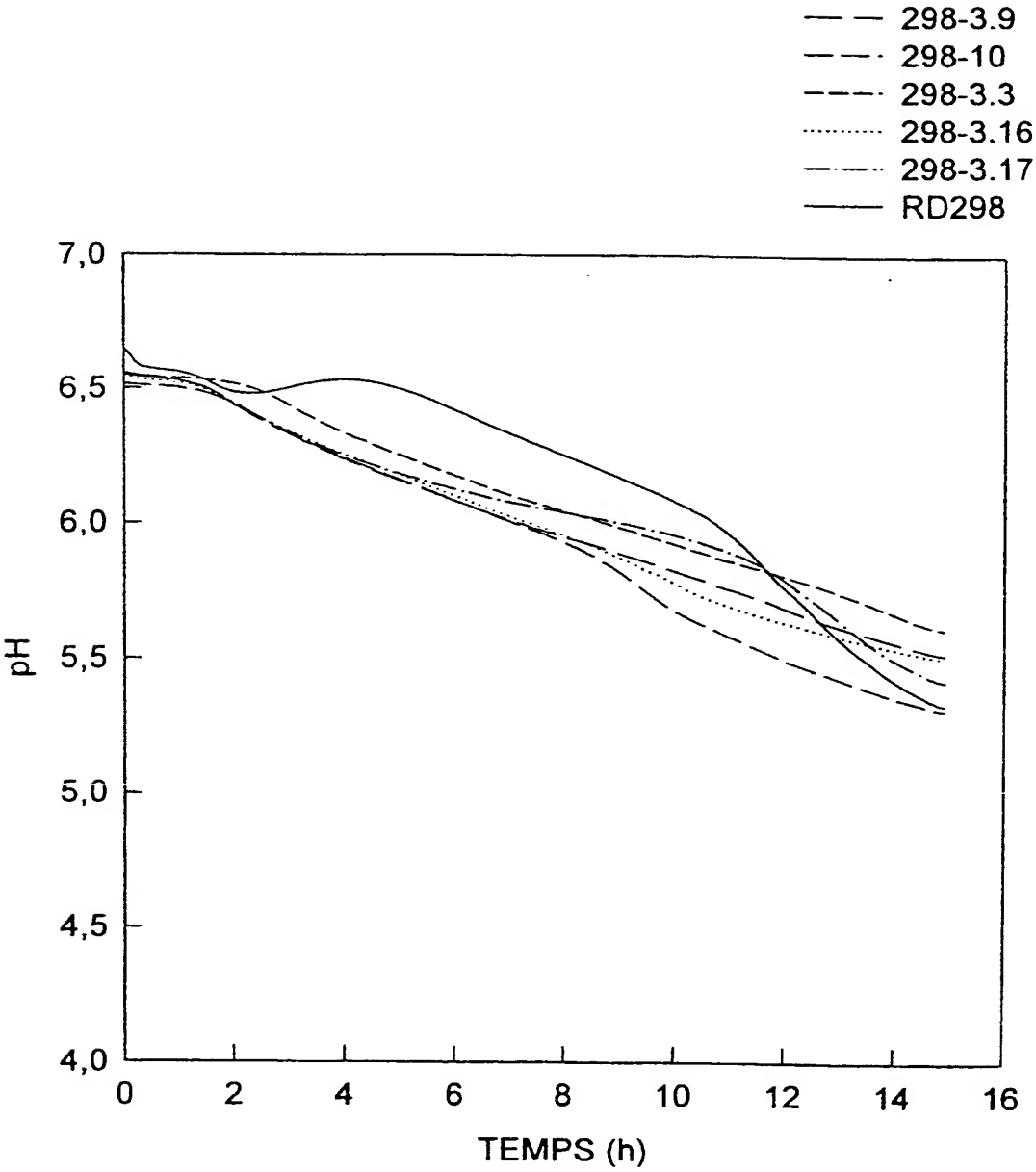
2
4
6
8
10
12
14
16
TEMPS (h)

FIG.1A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298-3.3
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4,0
pH
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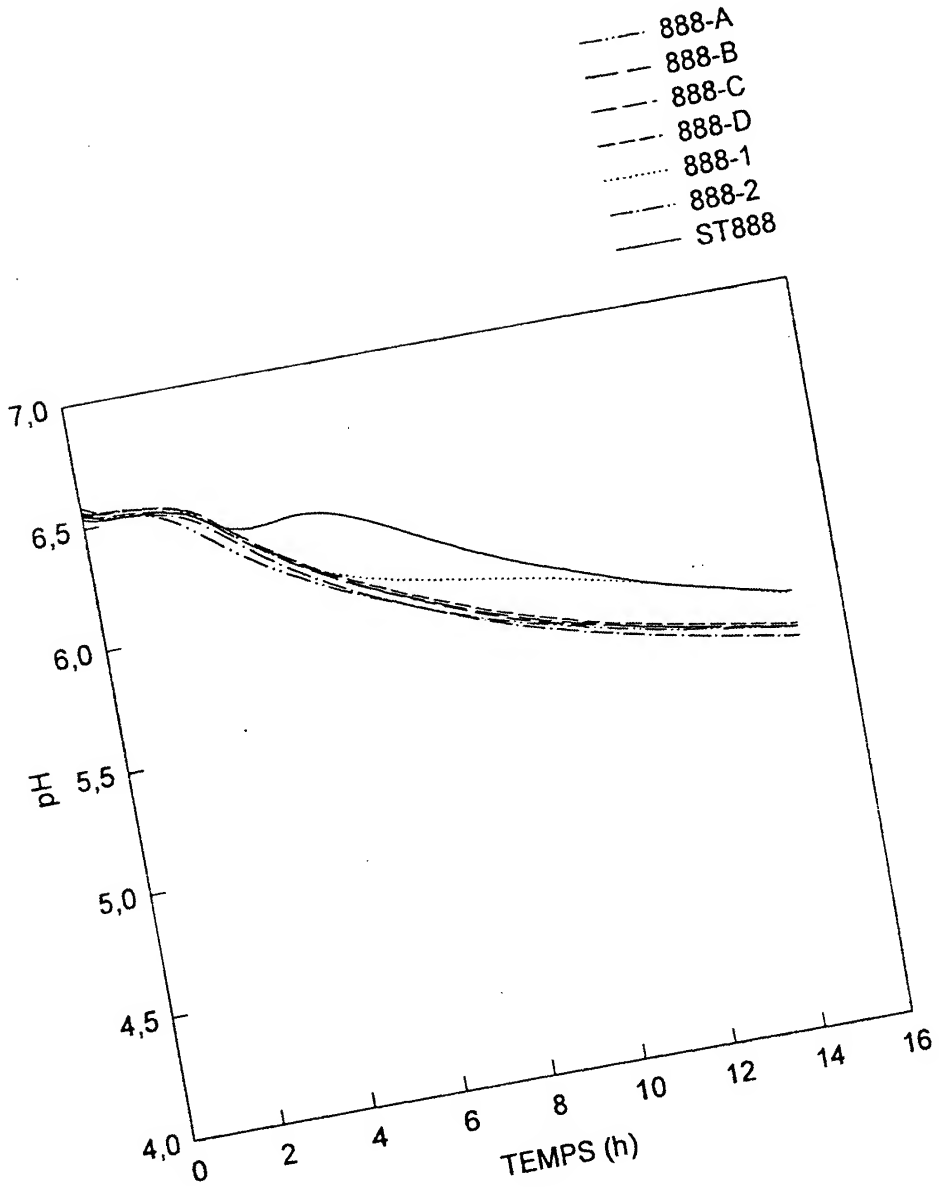
6
8
10
12
14
16
TEMPS (h)

FIG.1B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888-C
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888-2
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5,0
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4,0
pH
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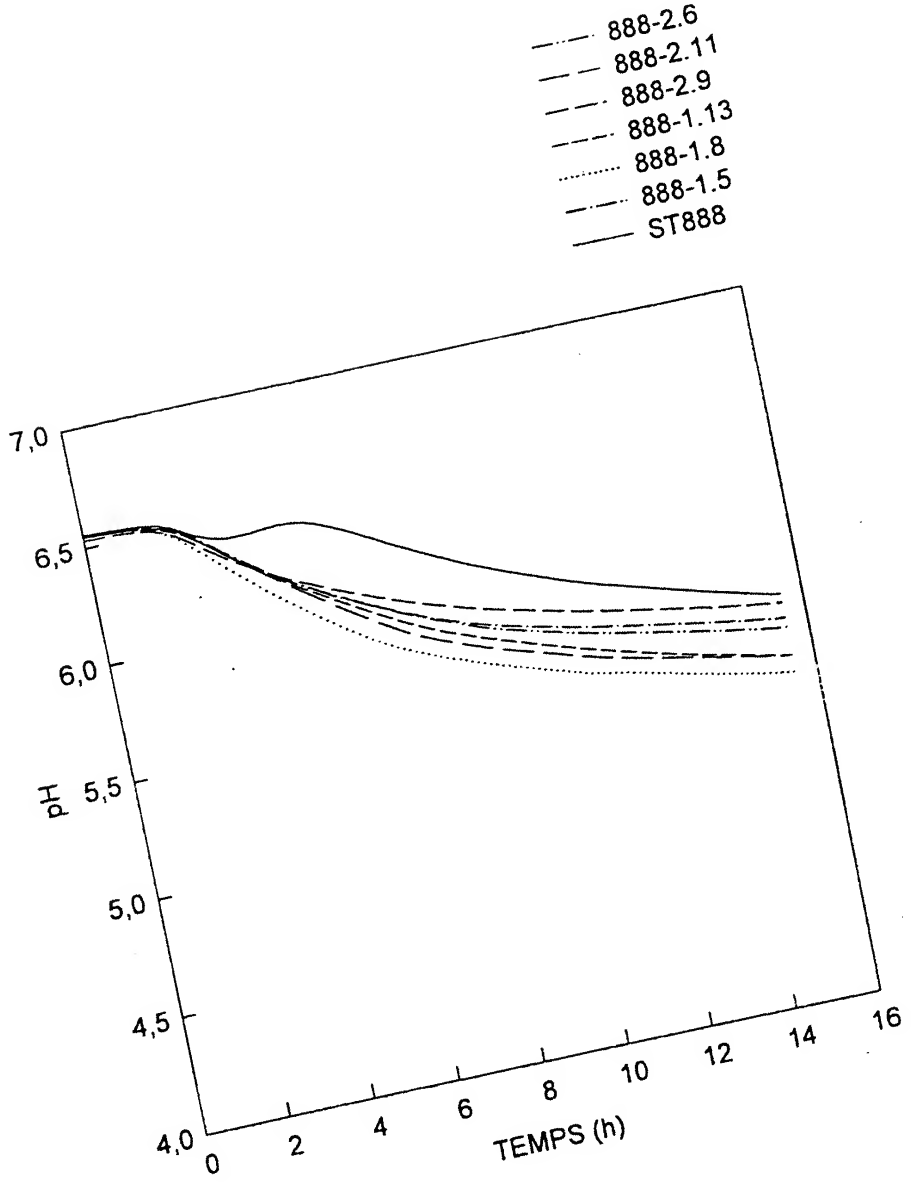
2
4
6
8
10
12
14
16
TEMPS (h)

FIG.2A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888-1.5
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6,0
5,5
5,0
4,5
4,0
pH
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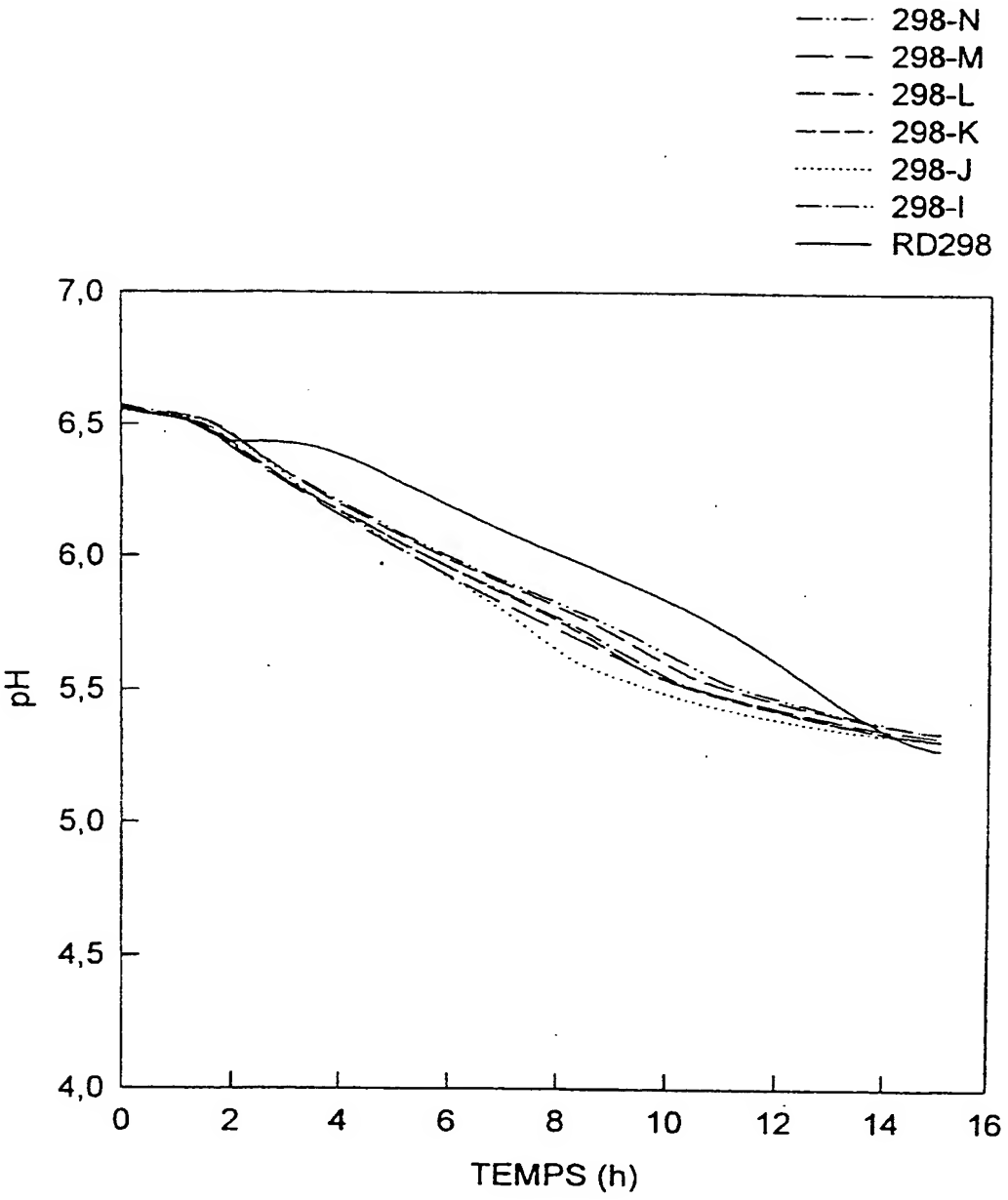
2
4
6
8
10
12
14
16
TEMPS (h)

FIG.2B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298-K
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6,0
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5,0
4,5
4,0
pH
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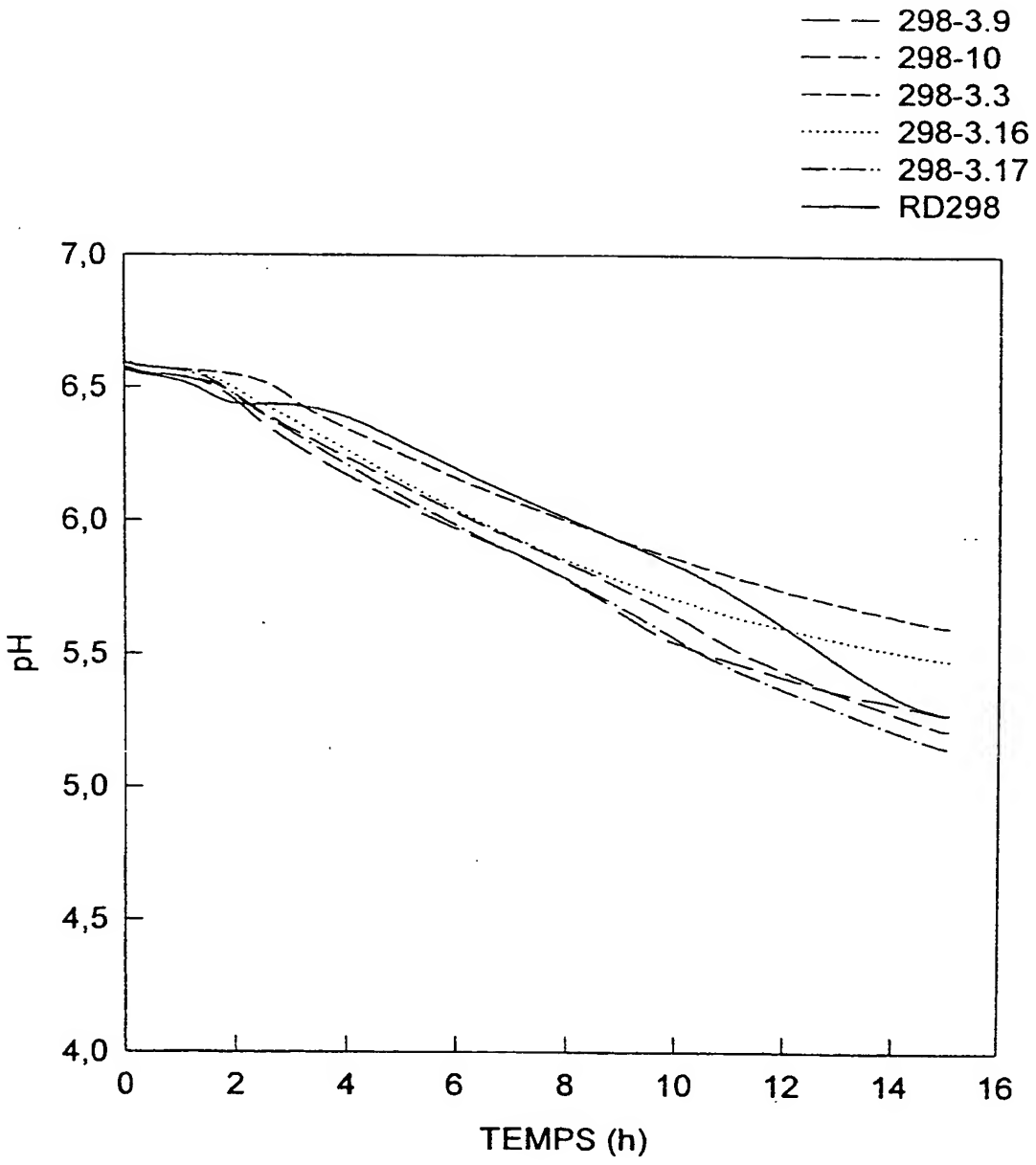
2
4
6
8
10
12
14
16
TEMPS (h)

FIG.3A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4,0
pH
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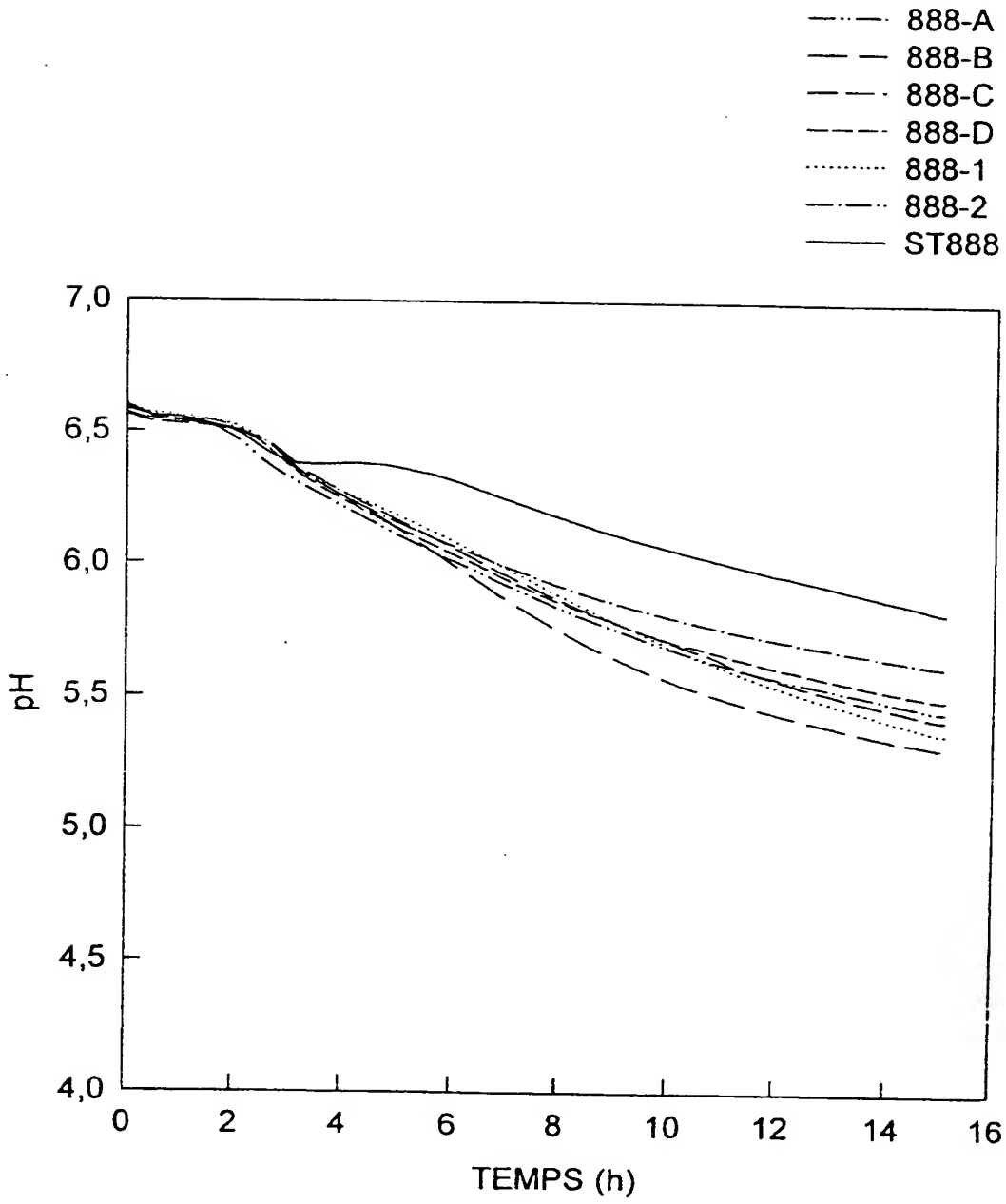
6
8
10
12
14
16
TEMPS (h)

FIG.3B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888-C
888-D
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888-2
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pH
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8
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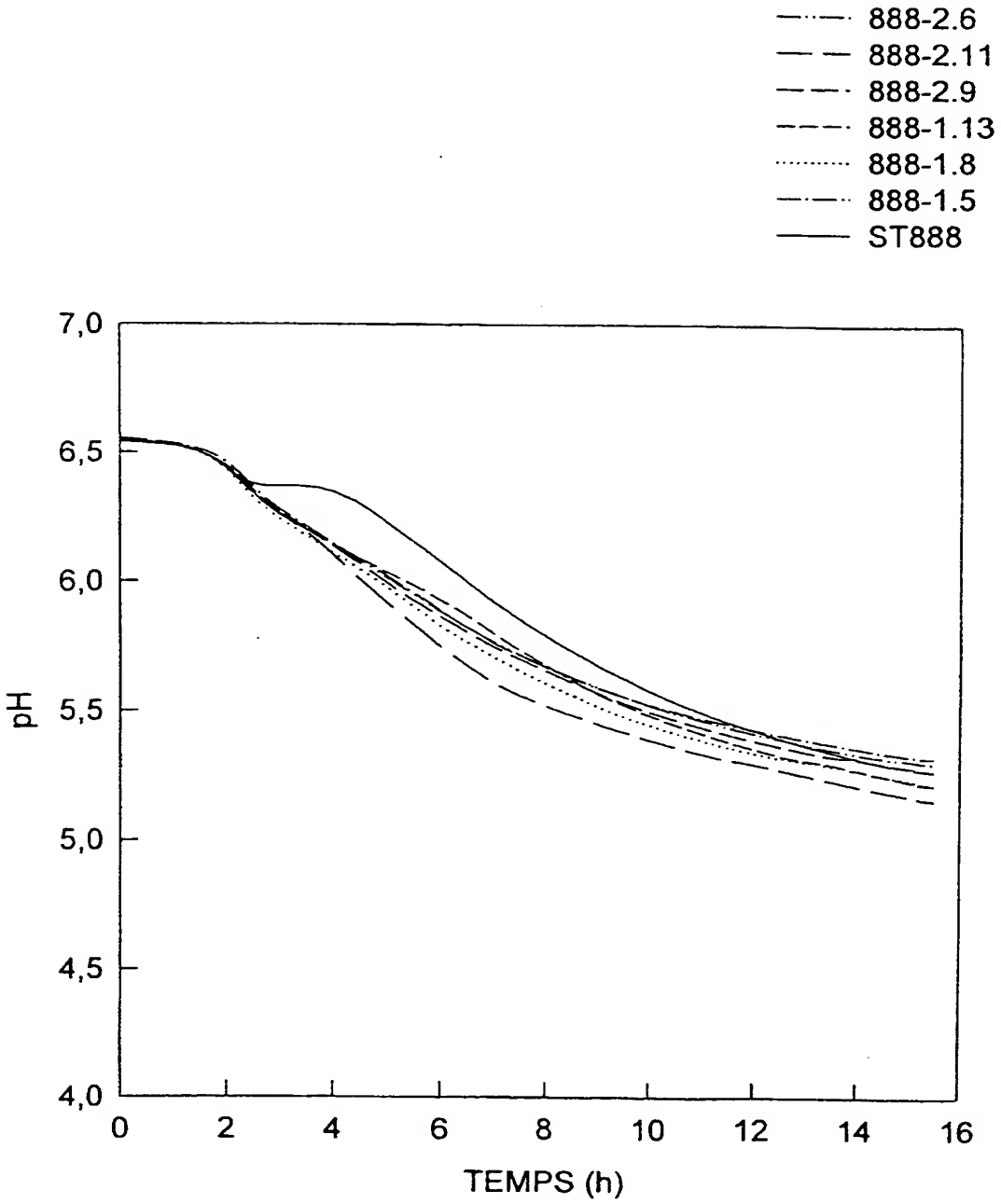
16
TEMPS (h)

FIG.4A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888-2.9
888-1.13
888-1.8
888-1.5
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6,0
5,5
5,0
4,5
4,0
pH
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2
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6
8
10
12
14
16
TEMPS (h)

FIG.4B

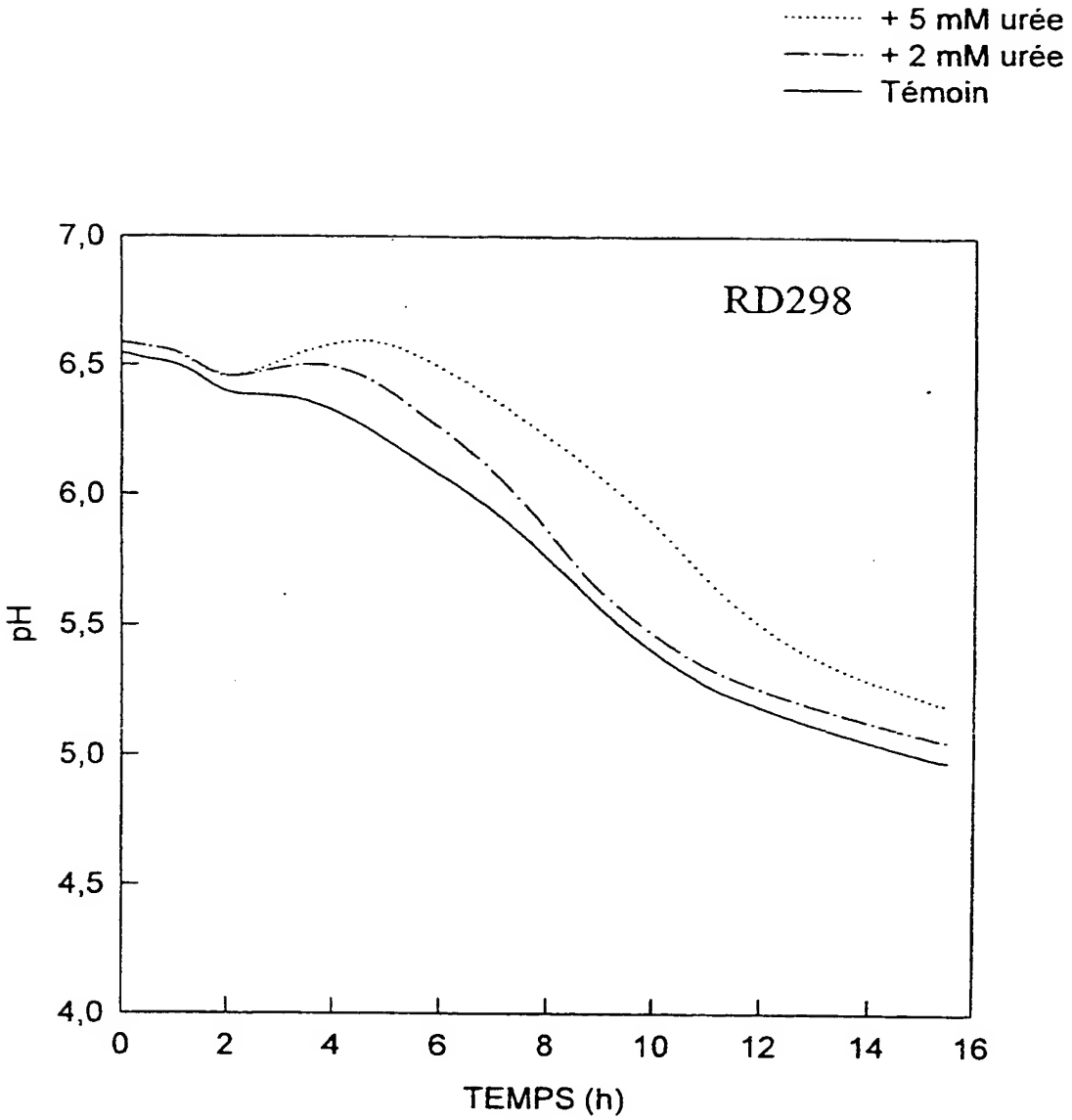
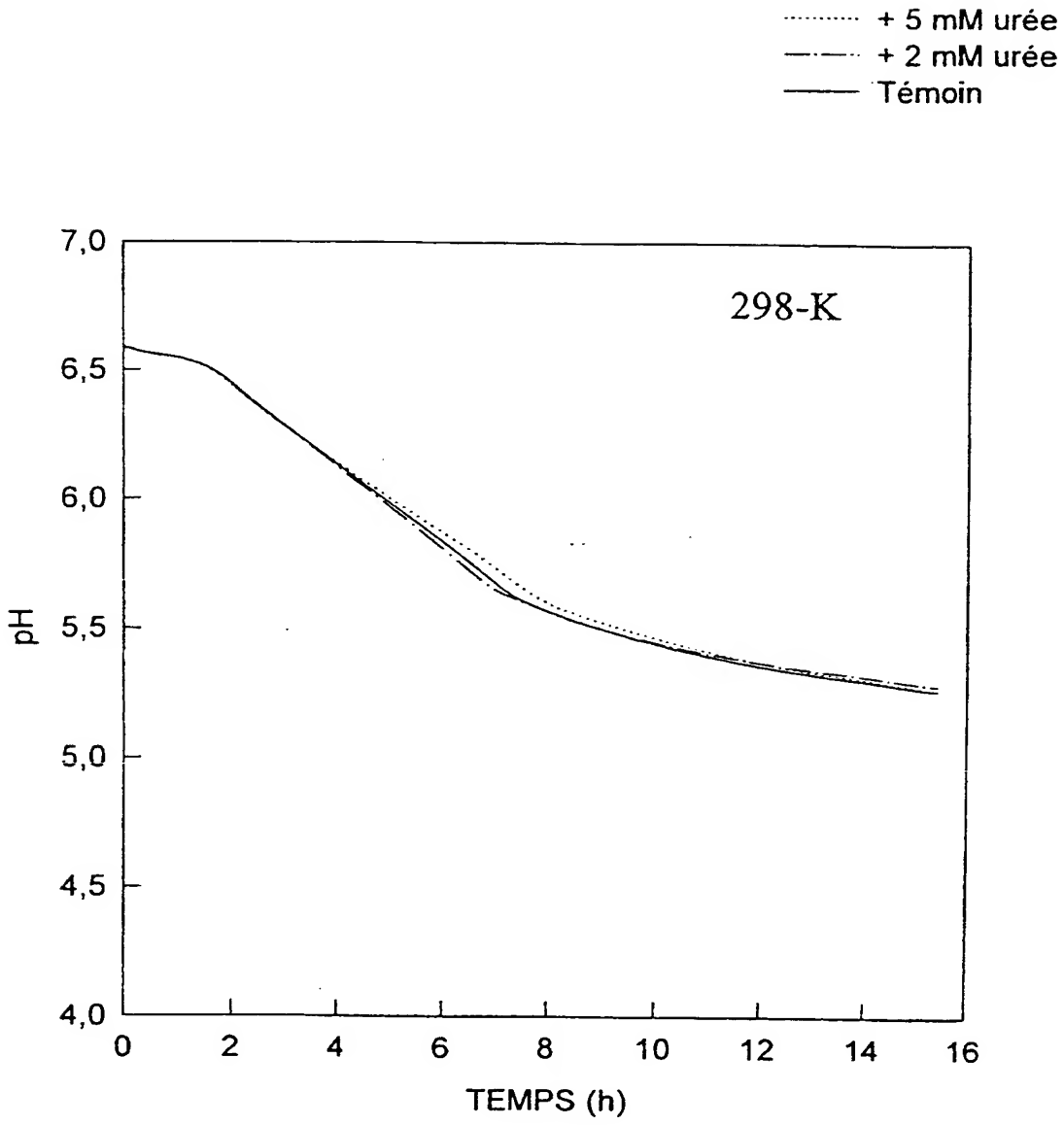

+ 5 mM urée
+ 2 mM urée
Témoin
RD298
7,0
6,5
6,0
5,5
5,0
4,5
4,0
pH
0
2
4
6
8
10
12
14
16
TEMPS (h)

FIG.5A


+ 5 mM urée
+ 2 mM urée
Témoin
298-K
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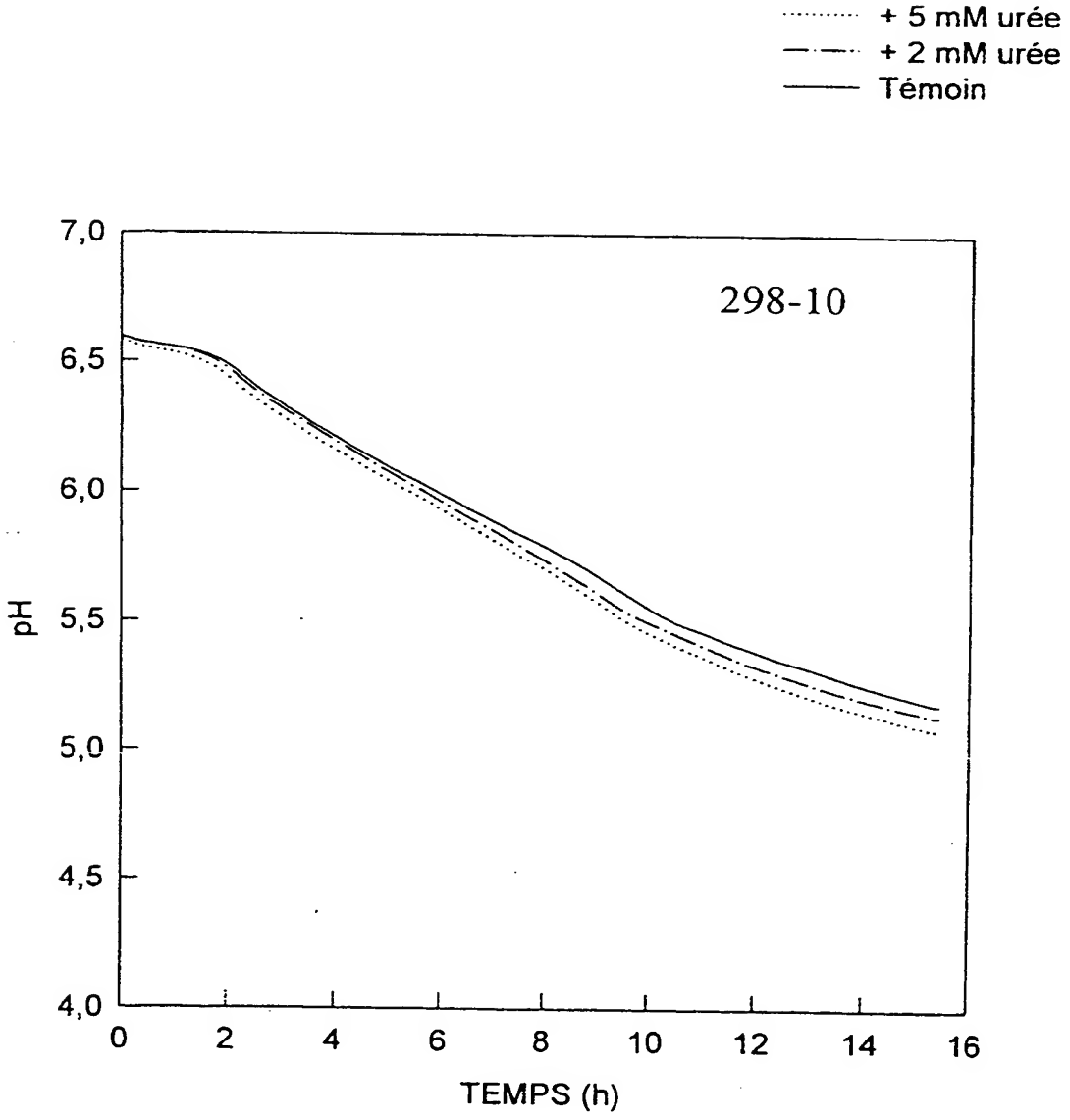
6,5
6,0
5,5
5,0
4,5
4,0
pH
0
2
4
6
8
10
12
14
16
TEMPS (h)

FIG.5B


+ 5 mM urée
+ 2 mM urée
Témoin
298-10
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6,5
6,0
5,5
5,0
4,5
4,0
pH
0
2
4
6
8
10
12
14
16
TEMPS (h)

FIG.5C

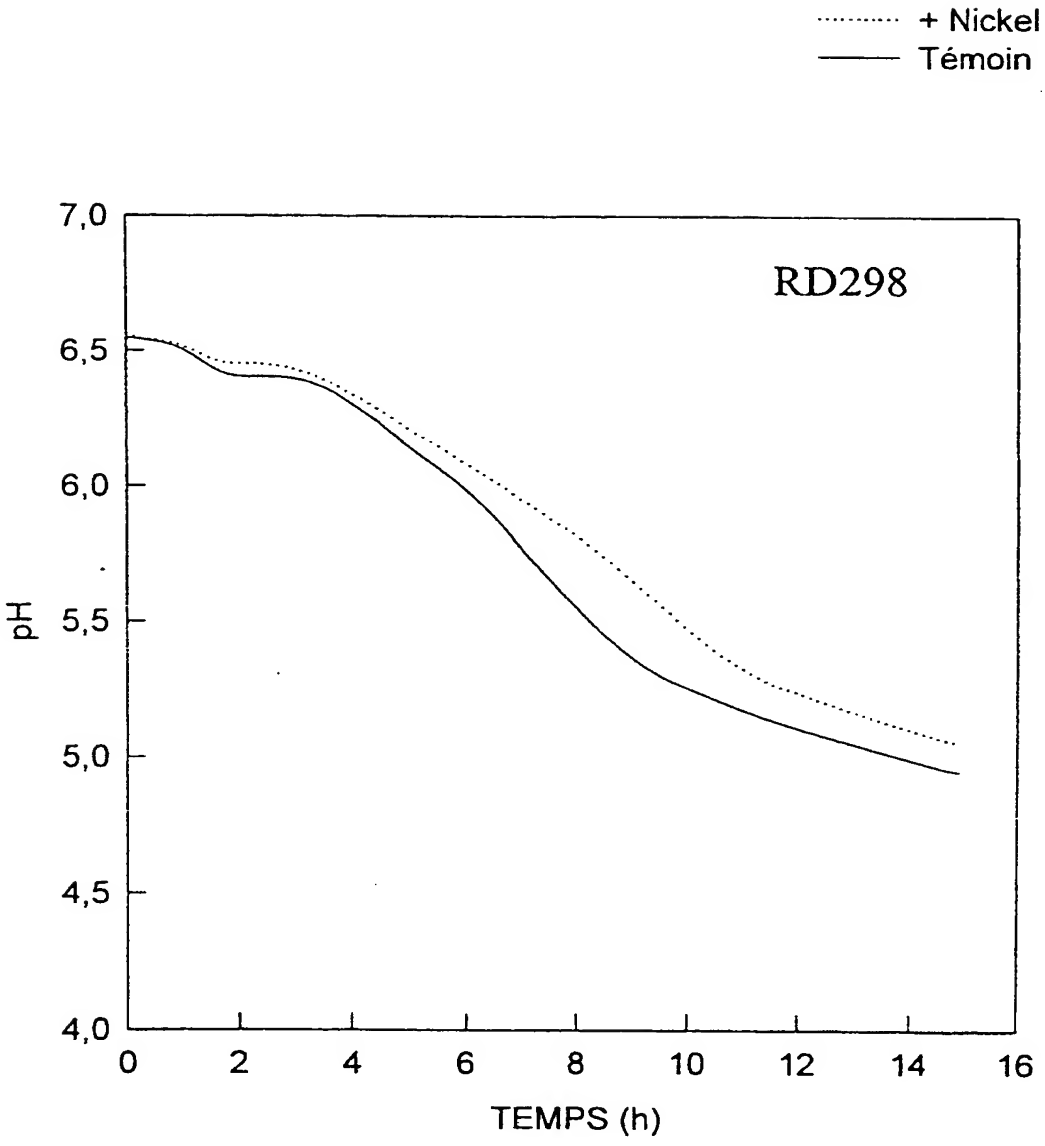
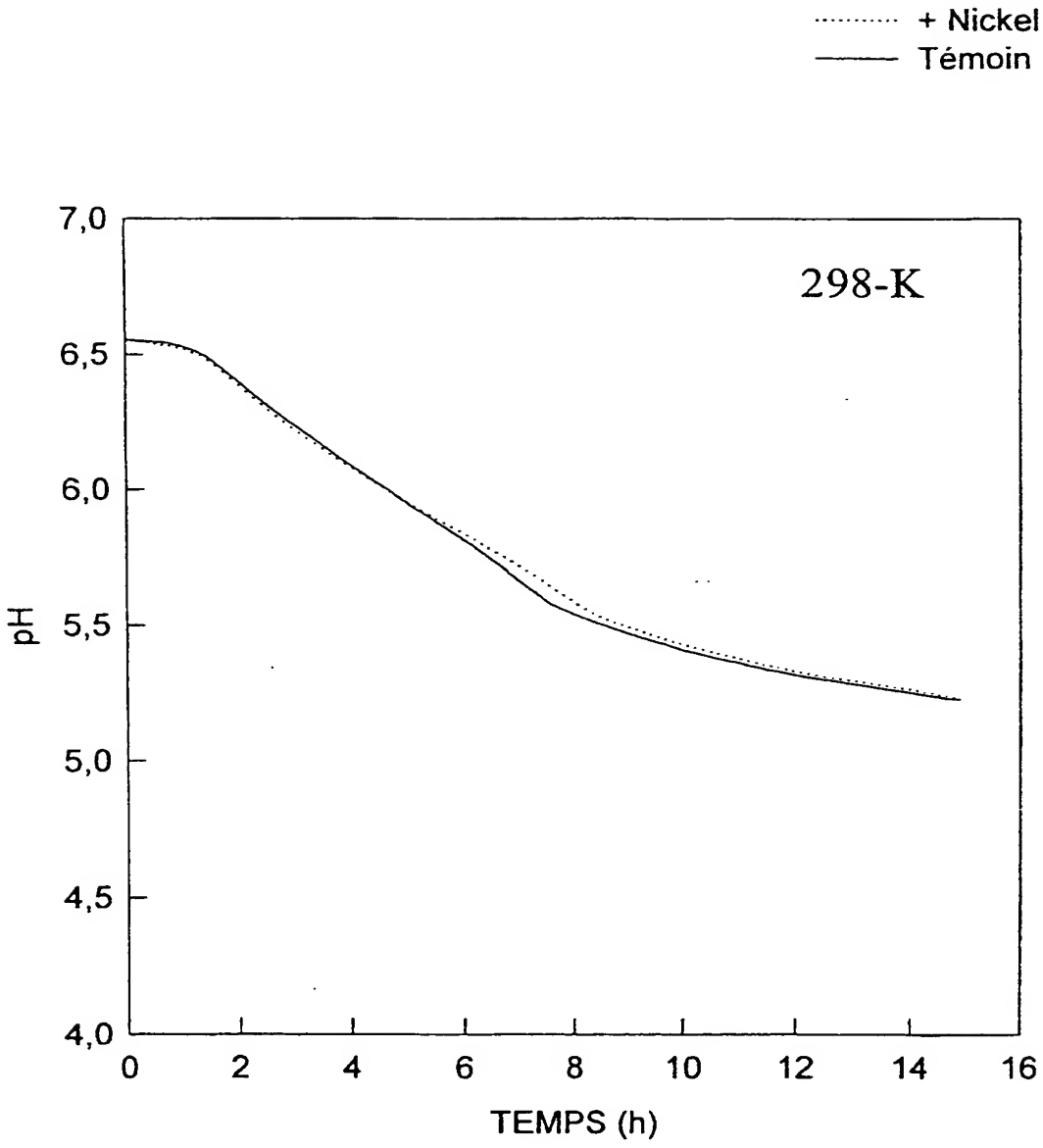

+ Nickel
Témoin
RD298
7,0
6,5
6,0
5,5
5,0
4,5
4,0
pH
0
2
4
6
8
10
12
14
16
TEMPS (h)

FIG.6A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Témoin
298-K
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6,5
6,0
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pH
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2
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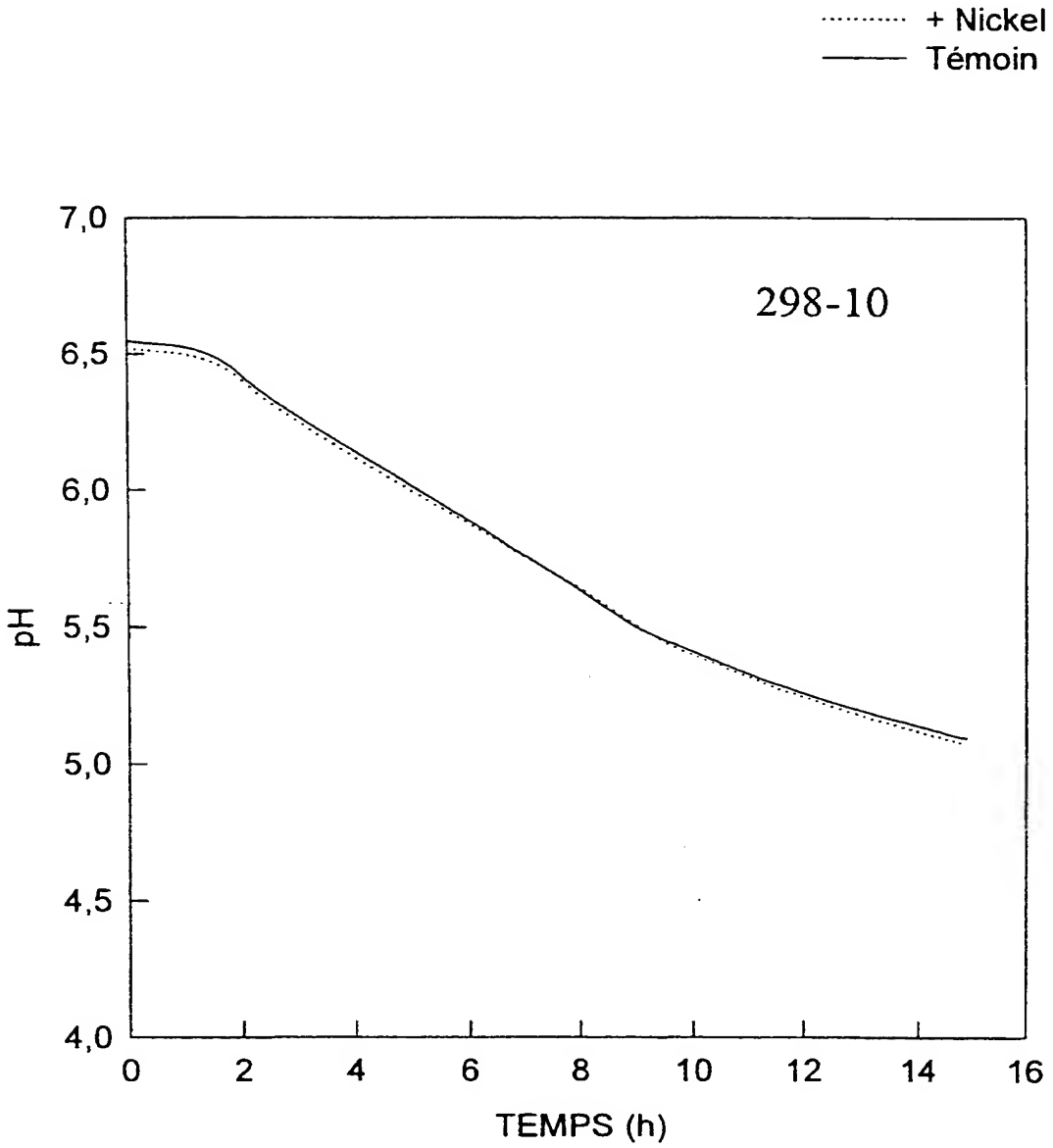
6
8
10
12
14
16
TEMPS (h)

FIG.6B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Témoin
298-10
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6
8
10
12
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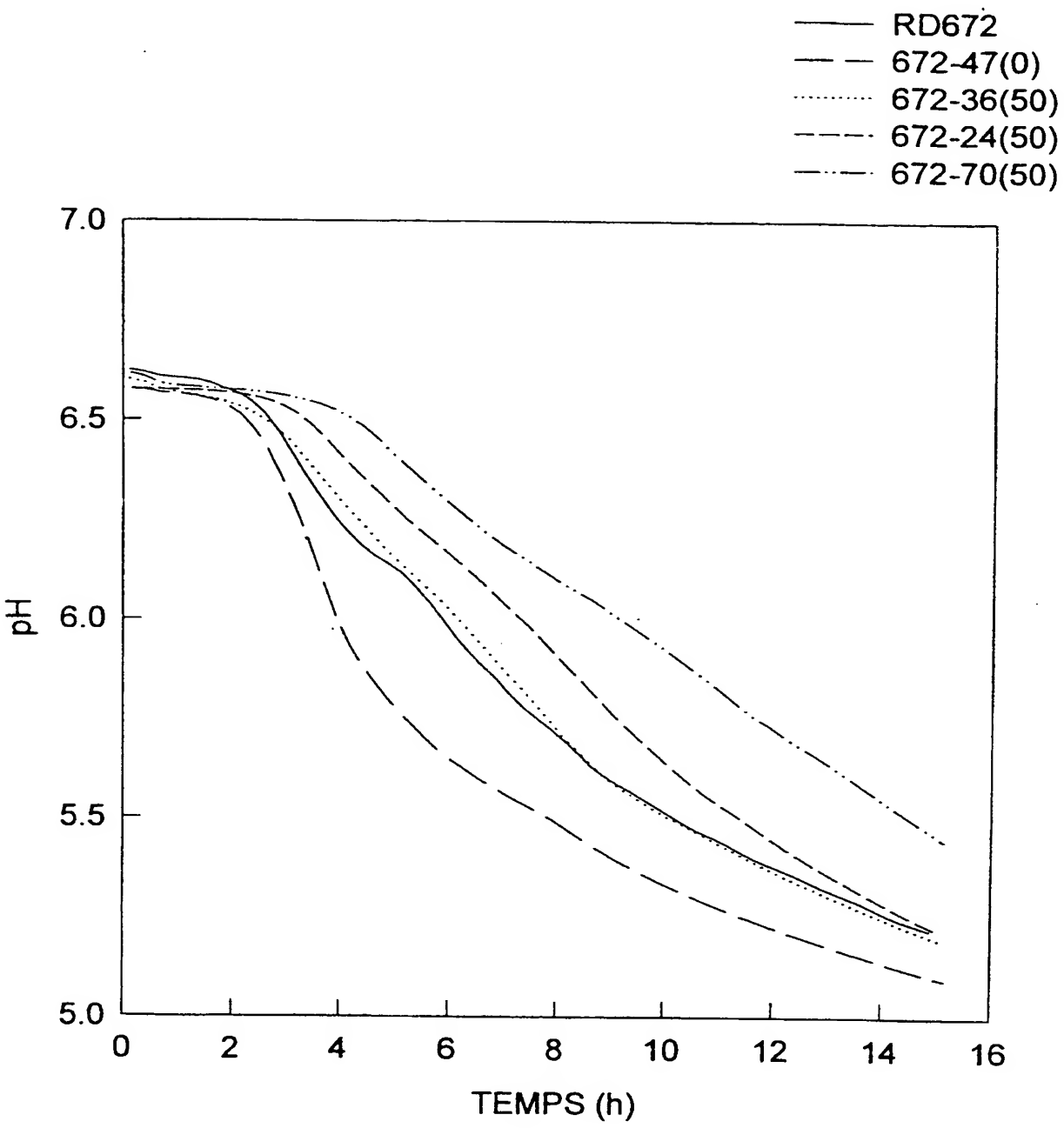
16
TEMPS (h)

FIG.6C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672-36(50)
672-24(50)
672-70(50)
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pH
0
2
4
6
8
10
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14
16
TEMPS (h)

FIG.7

INTERNATIONAL SEARCH REPORT

International Application No
PCT/FR 00/02577

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23C9/123 A23C19/032

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, EPO-Internal, FSTA, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	W. TINSON: "Metabolism of streptococcus thermophilus" THE AUSTRALIAN JOURNAL OF DAIRY TECHNOLOGY, vol. 37, no. 1, 1982, pages 17-21, XP002141061 cited in the application page 17 -page 20; table 1 ---	1,2,4,7, 9
X	B. BIANCHI SALVADORI: "Characteristics of some streptococcus thermophilus strains for the preparation of starters dehydrated for direct inoculation in cheese-vats" SCIENZA E TECNICA LATTIERO-CASEARIA, vol. 34, no. 4, 1983, pages 227-248, XP000920986 tables 2,4 --- -/--	1,2,7,9

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 December 2000	02/01/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Desmedt, G

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	A. ZOURARI: "Caractérisation de bactéries lactiques thermophiles isolées de yaourts artisanaux grecs" LE LAIT, vol. 77, no. 4, 1991, pages 445-461, XP000921064 page 450, column 1; figure 4 ---	7,9
A	WO 96 10627 A (GERVAIS DANONE CO ;BENBADIS LAURENT (FR); OUDOT ELISABETH (FR); VI) 11 April 1996 (1996-04-11) page 2, line 15 - line 18; claims 1-11 ---	1,7,9
A	V. JUILLARD: "Mise en évidence d'une activité uréasique chez Streptococcus thermophilus" CANADIAN JOURNAL OF MICROBIOLOGY, vol. 34, no. 6, 1988, pages 818-822, XP000921155 cited in the application page 818, column 1; table 1 -----	1,7,9

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internatic Application No
PCT/FR 00/02577

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9610627 A	11-04-1996	FR 2725212 A	05-04-1996
		AT 173011 T	15-11-1998
		DE 69505836 D	10-12-1998
		DE 69505836 T	27-05-1999
		DK 783566 T	19-07-1999
		EP 0783566 A	16-07-1997
		ES 2125050 T	16-02-1999
		US 6056979 A	02-05-2000

RAPPORT DE RECHERCHE INTERNATIONALE

Demande internationale No
PCT/FR 00/02577

A. CLASSEMENT DE L'OBJET DE LA DEMANDE
CIB 7 A23C9/123 A23C19/032

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Catégorie °	Identification des documents cités, avec, le cas échéant, l'indication des passages pertinents	no. des revendications visées
X	W. TINSON: "Metabolism of streptococcus thermophilus" THE AUSTRALIAN JOURNAL OF DAIRY TECHNOLOGY, vol. 37, no. 1, 1982, pages 17-21, XP002141061 cité dans la demande page 17 -page 20; tableau 1 ---	1,2,4,7, 9
X	B. BIANCHI SALVADORI: "Characteristics of some streptococcus thermophilus strains for the preparation of starters dehydrated for direct inoculation in cheese-vats" SCIENZA E TECNICA LATTIERO-CASEARIA, vol. 34, no. 4, 1983, pages 227-248, XP000920986 tableaux 2,4 --- -/--	1,2,7,9

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RAPPORT DE RECHERCHE INTERNATIONALE

Demande internationale No
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C.(suite) DOCUMENTS CONSIDERES COMME PERTINENTS

Catégorie °	Identification des documents cités, avec,le cas échéant, l'indicationdes passages pertinents	no. des revendications visées
X	A. ZOURARI: "Caractérisation de bactéries lactiques thermophiles isolées de yaourts artisanaux grecs" LE LAIT, vol. 77, no. 4, 1991, pages 445-461, XP000921064 page 450, colonne 1; figure 4 ---	7,9
A	WO 96 10627 A (GERVAIS DANONE CO ;BENBADIS LAURENT (FR); OUDOT ELISABETH (FR); VI) 11 avril 1996 (1996-04-11) page 2, ligne 15 - ligne 18; revendications 1-11 ---	1,7,9
A	V. JUILLARD: "Mise en évidence d'une activité uréasique chez Streptococcus thermophilus" CANADIAN JOURNAL OF MICROBIOLOGY, vol. 34, no. 6, 1988, pages 818-822, XP000921155 cité dans la demande page 818, colonne 1; tableau 1 -----	1,7,9

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RAPPORT DE RECHERCHE INTERNATIONALE

Renseignements relatifs aux membres de familles de brevets

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Document brevet cité au rapport de recherche	Date de publication	Membre(s) de la famille de brevet(s)	Date de publication
WO 9610627 A	11-04-1996	FR 2725212 A	05-04-1996
		AT 173011 T	15-11-1998
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		ES 2125050 T	16-02-1999
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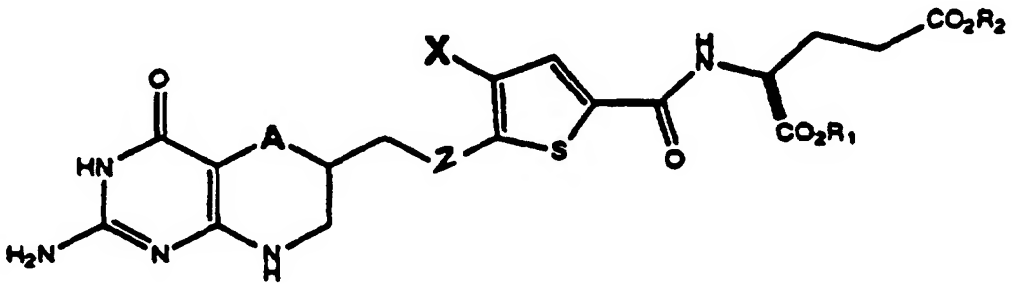

(I)

(57) Abstract

Compounds of formula (I), which are in equilibrium with their 4-hydroxy tautomers and are in the form of diastereomeric mixtures, and their pharmaceutically acceptable salts are potent GARFT inhibitors. A is S, CH_2 or Se; Z is a substituted or unsubstituted C_1-C_3 alkyl, C_2-C_3 alkenyl, C_2-C_3 alkynyl or amino group, or S or O; X is a substituted or unsubstituted C_1-C_6 alkyl group; a substituted or unsubstituted C_2-C_6 alkynyl group; a substituted or unsubstituted C_2-C_6 alkynyl group; -C(O)E, wherein E is hydrogen, a substituted r unsubstituted C_1-C_3 alkyl group, a substituted or unsubstituted C_2-C_3 alkenyl group, a substituted or unsubstituted C_2-C_3 alkynyl group, a substituted or unsubstituted OC_1-C_3 alkoxy group, or $NR_{10}R_{11}$, wherein R_{10} and R_{11} are independently selected from hydrogen, substituted and unsubstituted C_1-C_3 alkyl groups, substituted and unsubstituted C_2-C_3 alkenyl groups, substituted and unsubstituted C_2-C_3 alkynyl groups; $NR_{10}R_{11}$, wherein R_{10} and R_{11} are independently defined as set forth above; hydroxyl; nitro; SR_{12}, wherein R_{12} is hydrogen, a substituted or unsubstituted C_1-C_6 alkyl group, a substituted or unsubstituted C_2-C_6 alkenyl group, or a substituted or unsubstituted C_2-C_6 alkynyl group; cyano; r a substituted or unsubstituted C_1-C_3 alkoxy group; and R_1 and R_2 are independently hydrogen or a moiety that forms with the attached CO_2 a readily hydrolyzable ester group. These compounds and their salts are useful as antiproliferative agents. The invention also pertains to pharmaceutical compositions and methods employing such compounds as GARFT inhibitors or antiproliferative agents. The invention also relates to compounds useful as intermediates for preparing such compounds, and to their synthesis.

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COMPOUNDS USEFUL AS ANTIPROLIFERATIVE AGENTS AND GARFT INHIBITORS

BACKGROUND OF THE INVENTION

The present invention relates to compounds of the Formula I defined below, which inhibit the enzyme glycinamide ribonucleotide formyl transferase (GARFT). The invention also relates to pharmaceutical compositions containing the compounds of the Formula I, to their use to inhibit GARFT and to their use to inhibit the growth and proliferation of the cells of higher organisms or microorganisms such as bacteria, yeast and fungi. The invention also relates to the preparation of these compounds, and to intermediates used in their preparation.

GARFT is a folate dependent enzyme in the *de novo* purine biosynthesis pathway. This pathway is critical to cell division and proliferation. Shutting down this pathway is known to have an antiproliferative effect, in particular, an antitumor effect. Thus, a number of folate analogs have been synthesized and studied for their ability to inhibit GARFT. A prototypical specific tight-binding inhibitor of GARFT, 5,10-dideazatetrahydrofolic acid (DDATHF), has been reported to show antitumor activity. See F.M. Muggia, "Folate antimetabolites inhibitor to *de novo* purine synthesis," *New Drugs, Concepts and Results in Cancer Chemotherapy*, Kluwer Academic Publishers, Boston (1992), 65-87.

The large class of antiproliferative agents includes antimetabolite compounds. A particular subclass of antimetabolites known as antifolates or antifoles are antagonists of the vitamin folic acid. Typically, antifolates closely resemble the structure of folic acid and incorporate the characteristic P-benzoyl glutamate moiety of folic acid. The glutamate moiety of folic acid takes on a double negative charge at physiological pH, and therefore this compound and its analogs have an active energy driven transport system to cross the cell membrane and exert a metabolic effect. Research by a number of investigators has show that folic acid in both its reduced

and oxidized forms and its analogs are actively transported into cells by at least two distinct transport mechanisms. These transport proteins are referred to as the reduced folate transport protein, which has a preference for reduced folates but will transport a number of folic acid derivatives. Methotrexate (MTX) is transported via the reduced folate transport system. The other folate transport protein is referred to as the membrane folate binding protein or mFBP, which has a preference for folic acid. See A. C. Antony, "The Biological Chemistry of Folate Receptors," *Blood, The Journal of the American Society of Hematology*, vol. 79 (1992), 2807-2820.

The anticancer glutamate-containing antifolates used clinically to date, including MTX, enter cells via the reduced folate transport system with one notable exception. 5,10-Dideaza-tetrahydrofolic acid (DDATHF) is an antitumor GARFT inhibitor currently undergoing clinical study. DDATHF has been shown to be transported into cells via both the reduced folate transport system and the mFBP. See G. Pizzorno et al., "5,10-Dideazatetrahydrofolic Acid (DDATHF) Transport in CCRF-CEM and MA104 Cell Lines," *The Journal of Biological Chemistry*, vol. 268 (1993), 1017-1023.

It has been suggested that undesirable toxicity, particularly in folate-depleted mammals, is related to the fact that DDATHF, a prior art GARFT inhibitor, has a high affinity for the mFBP, which is unregulated during times of folate deficiency. It has been further suggested that folic acid and other molecules that block the mFBP from transporting other GARFT inhibitors can attenuate the toxicity of such inhibitors. See, e.g., T. Alati et al., "Evaluation of the Mechanism(s) of Inhibition of the Toxicity, But Not the Antitumor Activity of Lometrexol (DDATHF) by Folic Acid," *Proceedings of the American Association for Cancer Research*, vol. 33 (1992), Abstract 2432, 407; L. L. Habeck et al., "A Novel Class of Monoglutamated Antifolates Exhibits Tight-binding Inhibition of Human Glycinamide Ribonucleotide

Formyltransferase and Potent Activity against Solid Tumors," *Cancer Research*, vol. 54 (1994), 1021-1026; and U.S. Patent 5,217,974 to Grindey et al.

Summary of the Invention

Thus, an object of this invention is to produce compounds that are potent GARFT inhibitors having reduced toxicity. This object has been achieved through the antiproliferative agents of the Formula I below that are potent GARFT inhibitors but do not have tight binding to the mFBP. These compounds preferably have binding constants to the mFBP of at least a factor of 1000 less than DDATHF, yet still retain the favorable properties of GARFT inhibition and reduced folate transport for antitumor activity.

As indicated above, compounds of the invention possess antiproliferative activity, a property which can express itself in the form of antitumor activity. A compound of the invention can be active *per se*, or as a precursor converted *in vivo* to an active compound. Preferred compounds of the invention are especially active in inhibiting the enzyme GARFT. Particularly preferred compounds are active in inhibiting the growth of the L1210 cell line, a mouse leukemia cell line that can be grown in tissue culture. Compounds of the invention can also be active in inhibiting the growth of bacteria such as *Escherichia coli* gram-negative bacteria which can be grown in culture.

The compounds according to the invention, as well as the pharmaceutically acceptable salts thereof, may be incorporated into convenient dosage forms, such as capsules, tablets and injectable preparations. Solid or liquid pharmaceutically acceptable carriers, diluents or excipients may also be employed.

Solid carriers include starch, lactose, calcium sulfate dihydrate, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate and stearic acid.

Liquid carriers include syrup, peanut oil, olive oil, saline solution and water.

The carrier or diluent may include any prolonged-release material, such as glyceryl monostearate or glyceryl distearate, alone or with wax. When a liquid carrier is used, the preparation may be in the form of a syrup, elixir, emulsion, soft gelatin capsule, sterile injectable liquid (e.g. solution) or a nonaqueous or aqueous liquid suspension.

The pharmaceutical preparations are prepared following conventional techniques of the pharmaceutical chemist involving steps such as mixing, granulation and compressing when necessary for tablet forms, or mixing, filling and dissolving the ingredients as appropriate to give the desired products for oral, parenteral, topical, intravaginal, intranasal, intrabronchial, intraocular, intraaural or rectal administration.

The compositions of the invention may further comprise one or more other pharmaceutically active compounds. For example, one of the following antitumor agents may be included in the composition: mitotic inhibitors (e.g., vinblastine); alkylating agents; dihydrofolate reductase inhibitors or TS inhibitors; antimetabolites (for example, 5-fluorouracil, cytosinerabinoside); intercalating antibiotics (for example, adriamycin, bleomycin); enzymes (for example, asparaginase); topoisomerase inhibitors (for example, etoposide); and biological response modifiers (for example, interferon). The compounds of the invention may also be used in combination with one or more antiproliferative agents or GARFT inhibitors, such as a compound described in commonly assigned International Publication No. WO 94/13295, published June 23, 1994, or International Publication No. WO 92/05153, published April 2, 1992, the disclosures of which are incorporated by reference herein. The compositions of the invention may also comprise one or more antibacterial, antifungal, antiparasitic, antiviral,

antipsoriatic or anticoccidial agents. Exemplary antibacterial agents include: sulfonamides, such as sulfamethoxazole, sulfadiazine, sulfameter and sulfadoxine; dihydrofolic reductase inhibitors, such as trimethoprim, bromodiaprim and trimetrexate; penicillins; cephalosporins; and the quinolone carboxylic acids and their fused isothiazolo analogs.

Another aspect of the invention relates to a therapeutic method of inhibiting the growth or proliferation of cells of higher organisms or microorganisms, which comprises administering to a host an effective amount or quantity of a compound according to the present invention. The compounds of the invention are particularly useful in the treatment of mammalian hosts, such as human hosts, and in the treatment of avian hosts. A particularly preferred therapeutic process comprises administering to a host an amount of a compound according to the present invention effective to inhibit GARFT.

Many of the antiproliferative compounds described herein and their pharmaceutically acceptable salts thereof can be employed in the therapeutic process of the invention. The compounds may be administered in the form of a pharmaceutically acceptable composition comprising a diluent or carrier as described above.

A dose of a composition contains at least an effective quantity of the active compound and preferably is made up of one or more pharmaceutical dosage units. An "effective quantity" means a quantity sufficient to inhibit the folate metabolic pathways and derive the beneficial effects therefrom, e.g., through administration of one or more of the pharmaceutical dosage units.

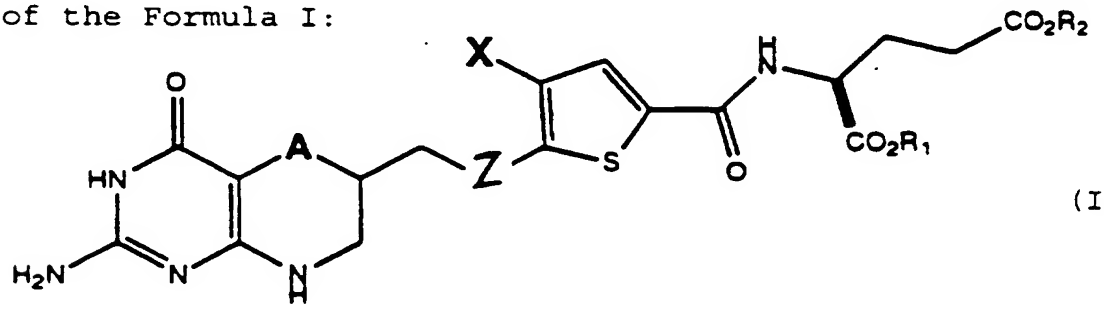
An exemplary daily dose for a vertebrate host comprises an amount of up to one gram active compound per kilogram of the host, preferably one-half of a gram, more preferably 100 milligrams, and most preferably, about 50 milligrams or less, per kilogram of the host's body weight. The selected dose may be administered to a warmblooded

animal or mammal, for example, a human patient in need of treatment mediated by folate metabolic pathways inhibition, by any suitable method of administrating the dose including: topically, for example, as an ointment or cream; orally; rectally, for example, as a suppository; parenterally by injection; or continuously by intravaginal, intranasal, intrabronchial, intraaural or intraocular infusion.

The compounds according to the invention produce any one or more of an antiproliferative effect, an antibacterial effect, an antiparasitic effect, an antiviral effect, an antipsoriatic effect, an antiprotozoal effect, an anticoccidial effect, an antiinflammatory effect, an immunosuppressive effect and an antifungal effect. The compounds are especially useful in producing an antitumor effect in a vertebrate host harboring a tumor.

Detailed Description of the Invention and Preferred Embodiments

In particular, the invention relates to compounds of the Formula I:



(I)

wherein:

A is sulfur, CH_2 or selenium;

Z is a substituted or unsubstituted C_1-C_3 alkyl group, a substituted or unsubstituted C_2-C_3 alkenyl group, a substituted or unsubstituted C_2-C_3 alkynyl group, a substituted or unsubstituted amino group, sulfur or oxygen;

X is a substituted or unsubstituted C_1-C_6 alkyl group; a substituted or unsubstituted C_2-C_6 alkenyl group; a substituted or unsubstituted C_2-C_6 alkynyl group; -C(O)E, wherein E is hydrogen, a substituted or unsubstituted C_1-C_3 alkyl group, a substituted or unsubstituted C_2-C_3 alkenyl

group, a substituted or unsubstituted C_2-C_3 alkynyl group, a substituted or unsubstituted OC_1-C_3 alkoxy group, or $NR_{10}R_{11}$, wherein R_{10} and R_{11} are independently selected from hydrogen, substituted and unsubstituted C_1-C_3 alkyl groups, substituted and unsubstituted C_2-C_3 alkenyl groups, substituted and unsubstituted C_2-C_3 alkynyl groups; $NR_{10}R_{11}$, wherein R_{10} and R_{11} are independently defined as set forth above; hydroxyl; nitro; SR_{12}, wherein R_{12} is hydrogen, a substituted or unsubstituted C_1-C_6 alkyl group, a substituted or unsubstituted C_2-C_6 alkenyl group, or a substituted or unsubstituted C_2-C_6 alkynyl group; cyano; or a substituted or unsubstituted $O(C_1$-$C_3)$ group; and

R_1 and R_2 are each independently hydrogen or a moiety that forms (together with the attached CO_2) a readily hydrolyzable ester group.

The invention also relates to pharmaceutically acceptable salts of the compounds of Formula I.

Although the compounds of the Formula I are shown in the 4-oxo form and are referred to as such throughout this description, the oxo group exists in tautomeric equilibrium with the corresponding 4-hydroxy group. It will therefore be understood that the compounds of the Formula I include the structurally depicted 4-oxo and the tautomeric 4-hydroxy forms. Thus, the invention also relates to pharmaceutically acceptable salts of the 4-hydroxy tautomers of the compounds depicted by Formula I.

The compounds of the Formula I are in the form of diastereomeric mixtures. It will be understood that unless indicated otherwise, the compounds having chiral centers are in the form of mixtures of diastereomers.

Preferably, A is sulfur or CH_2.

When Z is substituted, the substituents are preferably selected from C_{1-6} alkoxyl, C_{1-6} alkyl and C_{2-6} alkenyl such as vinyl, C_{2-6} alkynyl, acyl such as formyl and acetyl, halogen, amino, hydroxyl, nitro, mercapto, monocyclic carbocycle, monocyclic heterocycle, nonfused polycyclic carbocycle, nonfused polycyclic heterocycle,

hydroxy C_{1-6} alkyl such as hydroxymethyl, and C_{1-6} alkoxy C_{1-6} alkyl. Preferably, Z is CH_2, CH_2CH_2, NH, oxygen, sulfur, $CH(CH_2OH)$ or NCH_3. More preferably, Z is CH_2.

When X is substituted, the substituents are preferably selected from OH, NH_2, O-methyl, O-ethyl, SH, SCH_3 and NH-methyl. Preferably, X is a substituted or unsubstituted C_1-C_6 alkyl group. Also, X is preferably unsubstituted. More preferably, X is methyl or ethyl.

Preferably, R_1 and R_2 each is independently hydrogen, C_1-C_6 alkyl, hydroxyalkyl, alkylaryl or aralkyl. More preferably, R_1 and R_2 each is independently hydrogen or C_1-C_2 alkyl.

In particularly preferred embodiments, A is sulfur or CH_2, Z is CH_2, and X is methyl.

Preferred examples of compounds of the Formula I include:

N-(5-[2-(2-amino-4(3H)-oxo-5,6,7,8-tetrahydropyrido[2,3-d]-pyrimidin-6-yl)ethyl]-4-methylthieno-2-yl)-L-glutamic acid;

N-(5-[2-(2-amino-4-oxo-4,6,7,8-tetrahydro-3H-pyrimido[5,4-6][1,4]-thiazin-6-yl)ethyl]-4-methylthieno-2-yl)-L-glutamic acid diethyl ester; and

N-(5-[2-(2-amino-4-oxo-4,6,7,8-tetrahydro-3H-pyrimido[5,4-6][1,4]thiazin-6-yl)ethyl]-4-methylthieno-2-yl)-L-glutamic acid.

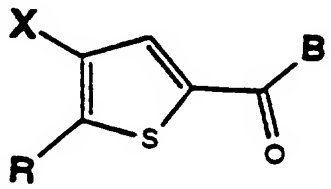
The compounds of the Formula I are useful as GARFT inhibitors. The compounds of Formula I in which R_1 and R_2 are each hydrogen are especially active antitumor or antiproliferative agents. The compounds of Formula I wherein R_1 and R_2 are each a moiety that forms a readily hydrolyzable ester group with the attached carboxyl, preferably an ethyl group, are useful intermediates for forming the free glutamic acid forms of the compounds and can also be hydrolyzed *in vivo* and thus act as prodrugs.

The pharmaceutically acceptable salts of the invention include, for example, alkaline metal, alkaline earth metal, other non-toxic metals, and ammonium and substituted ammonium salts of the glutamic acid compounds

of the invention. Exemplary salts include sodium, potassium, lithium, calcium, magnesium, pyridinium and substituted pyridinium salts of the free acid compounds.

The compounds of the Formula I can be prepared as described below.

To prepare compounds of the Formula I where Z is CH_2, a useful starting material is a compound of the Formula II:



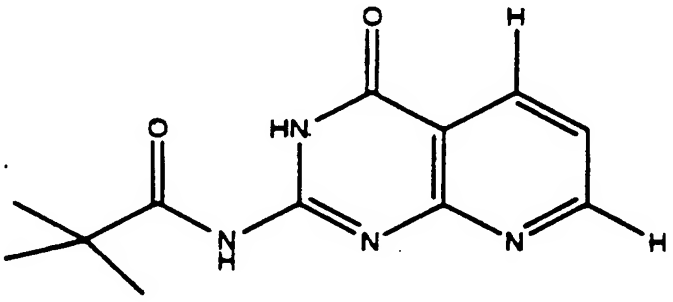
(II)

wherein: R is a halogen, preferably bromo; X is as defined above; and B is OH or an amino acid, preferably diethyl glutamate, linked through the amino portion to form an amide, or a C_1-C_6 alcohol, preferably a methyl or ethyl alcohol, linked through the alcohol portion to form an ester.

The compound of the Formula II is reacted with a compound of the Formula III:

(III)

wherein: Y is CH_2OH or a protected pyridopyrimidine of the Formula IV:

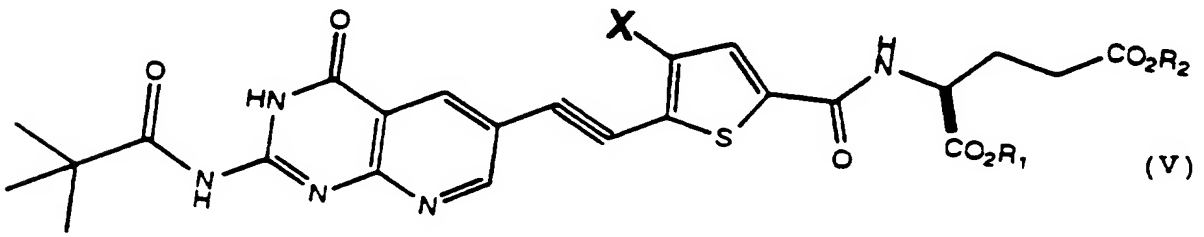


(IV)

The synthesis then can follow one of two routes, depending on whether Y is a protected pyridopyrimidine or CH_2OH.

Where Y is a protected pyridopyrimidine or CH_2OH of the Formula IV, the coupling reaction of compounds of the Formulae II and III is preferably conducted in the presence of a transition metal catalyst, preferably palladium or nickel, in the presence of a base, preferably a non-nucleophilic auxiliary base, in a solvent in which at least one of the reactants is at least partially soluble. Preferred solvents for the coupling reaction of the compounds of Formulae II and III are diethylamine, acetonitrile, dimethylformamide, dimethylacetamide and triethylamine. The basic medium for the coupling reaction is preferably provided via a non-nucleophilic auxiliary base, which is a base capable of neutralizing hydrogen halide acid generated by the coupling reaction. The base is preferably a di- or tri-alkylamine, such as diethylamine, triethylamine or diisopropylethylamine. Where appropriate, a basic solvent can be used instead of a separate solvent and base.

When Y is the pyridopyridimine the coupling reaction of the compounds of Formulae II and III produces a compound of the Formula V:

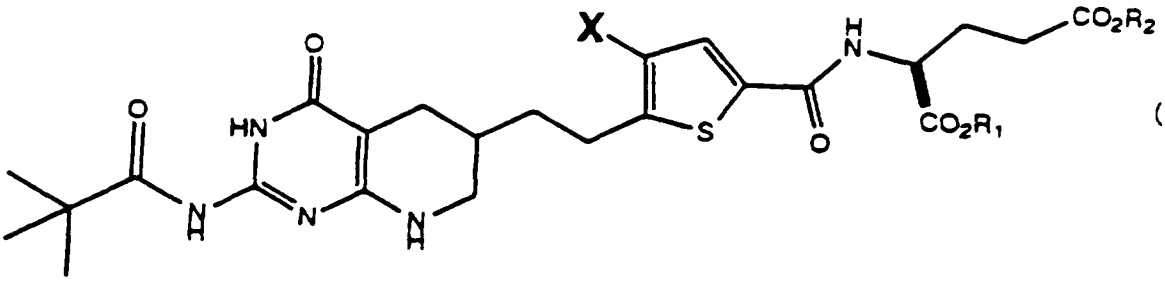


(V)

wherein X, R_1 and R_2 are as defined above.

The compound of the Formula V is reacted with hydrogen gas, preferably at 45-1000 psi, in the presence of a suitable transition metal catalyst, preferably platinum, palladium or rhodium metal on a carbon or other suitable support, in a suitable solvent, preferably acetic acid or

trifluoroacetic acid, to obtain a compound of the Formula VI:

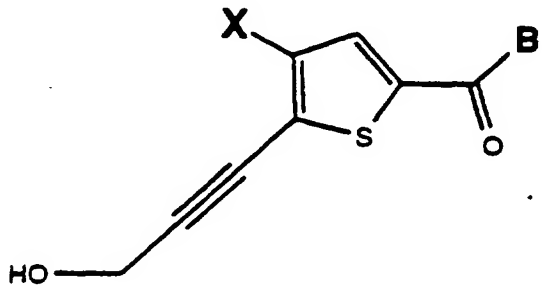


(VI)

wherein X, R_1, and R_2 are defined above.

Finally, the compound of Formula VI is hydrolyzed to form a free glutamic acid (R_1 and R_2 are each H) of Formula I.

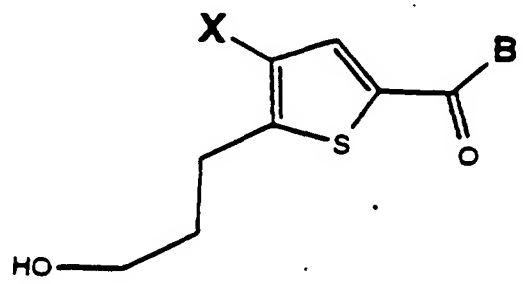
Where Y is CH_2OH, the reaction of the compounds of Formulae II and III produces a compound of the Formula VII:



(VII)

wherein X and B are as defined above.

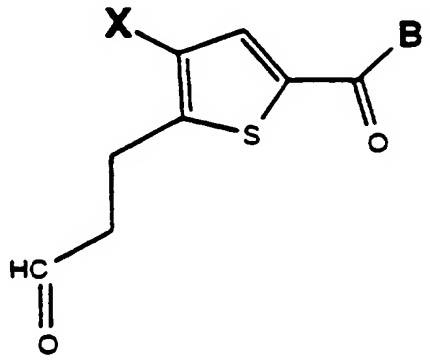
The compound of the Formula VII is reacted with hydrogen gas in the presence of a suitable metal catalyst, preferably palladium or platinum, to obtain a compound of the Formula VIII:



(VIII)

wherein X and B are as defined above.

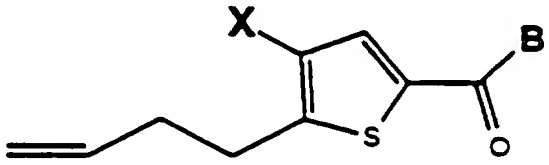
The compound of the Formula VIII is reacted with an oxidizing agent, preferably tetrapropylammonium perruthenate, to obtain a compound of the Formula IX:



(IX)

wherein X and B are as defined above.

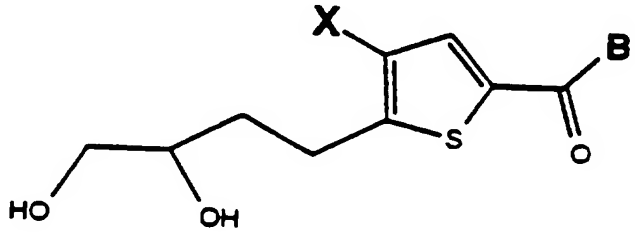
The compound of the Formula IX is reacted with a methylene transfer reagent, preferable methylene triphenylphosphorane, in a suitable solvent, preferably tetrahydrofuran, to obtain a compound of the Formula X:



(X)

wherein X and B are as defined above.

The compound of the Formula X is reacted with a dihydroxylating agent, preferably osmium tetroxide, in the presence of a suitable oxidizing agent, preferably N-methylmorpholine-N-oxide, to obtain a compound of the Formula XI:

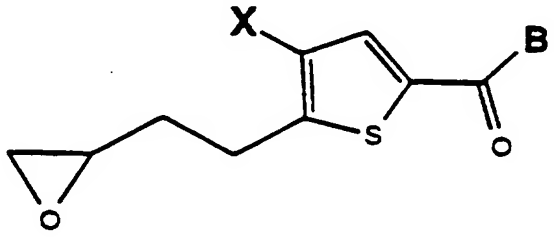


(XI)

wherein X and B are as defined above.

The compound of the Formula XI is converted to a compound of the Formula I using any of the four processes described below.

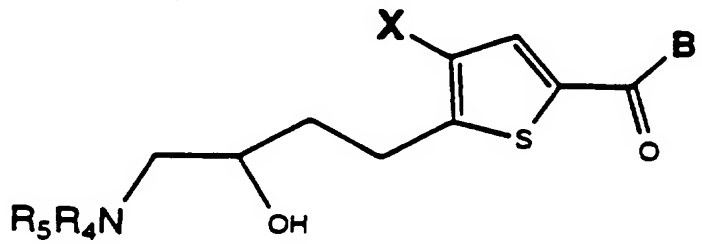
In a first conversion process, the compound of the Formula XI is reacted with a sulfonylating agent, preferably p-toluenesulfonyl chloride or methanesulfonyl chloride, in the presence of a non-nucleophilic base, preferably triethylamine or diisopropylethyl amine, to give an intermediate mono-sulfonylated compound. This intermediate is then reacted with a strong base, preferably sodium hydride, to obtain a compound of the Formula XII:



(XII)

wherein X and B are as defined above.

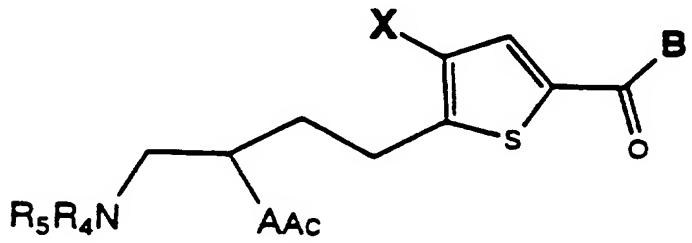
The epoxide of Formula XII is reacted with a nitrogen containing nucleophile, preferably sodium azide, in the presence of a mild Lewis-acid catalyst, preferably lithium perchlorate or magnesium perchlorate, to obtain an intermediate alcohol azide. Reduction of the alcohol azide, preferably with hydrogen gas in the presence of a metal catalyst, and subsequent protection with a suitable nitrogen-protecting group, preferably t-butoxycarbonyl, benzoxycarbonyl or benzyl, produces a compound of the Formula XIII:



(XIII)

wherein X and B are as defined above, and R_4 and R_5 are each independently hydrogen or a suitable nitrogen-protecting group. Preferred protecting groups are t-butoxycarbonyl, benzyl-oxycarbonyl and benzyl.

The compound of the Formula XIII is reacted with an acylating or sulfonylating agent, preferably methanesulfonyl chloride or p-toluenesulfonyl chloride, in the presence of a non-nucleophilic base, preferably triethylamine or diisopropylethylamine, in a suitable solvent in which at least one of the reactants is at least partially soluble, to obtain an activated hydroxy group. The activated hydroxy group is displaced with a suitable nucleophile, preferably a thioacid salt, more preferably potassium thioacetate, to obtain a compound of the Formula XIV:

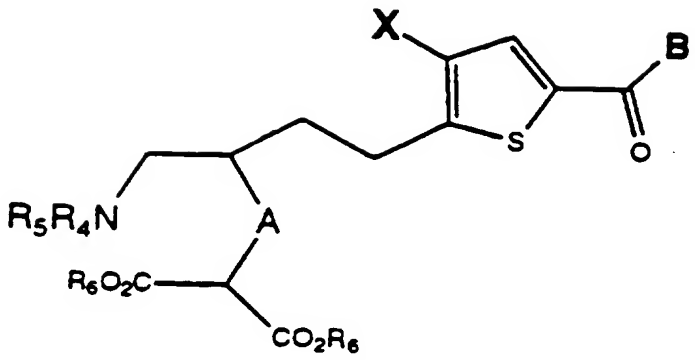


(XIV)

wherein A, X, B, and R_4 and R_5 are as defined above, and Ac is an acyl group. Preferably, Ac is acetyl.

Alternatively, the compound of the Formula XIII can be converted to the compound of the Formula XIV in one chemical operation using triphenylphosphine, diethyl or dimethyl azadicarboxylate, and an acidic nucleophile, preferably thioacetic acid, in a suitable solvent.

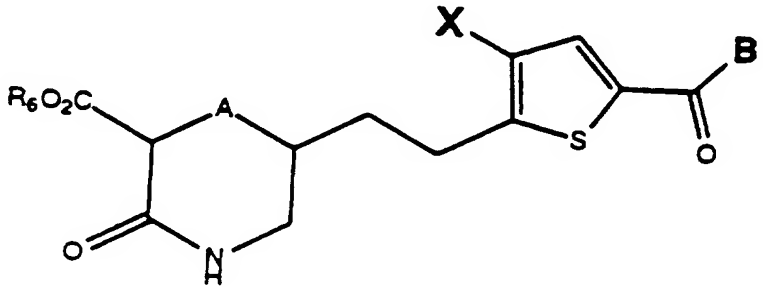
The compound of the Formula XIV is treated with a nucleophilic base, preferably potassium carbonate, sodium carbonate, sodium hydroxide or potassium hydroxide, in an alcoholic solvent, preferably methanol, ethanol or isopropanol, in the presence of an alkylating agent, preferably dimethyl or diethyl chloromalonate, to obtain a compound of the Formula XV:



(XV)

wherein A, X, B, and R_4 and R_5 are as defined above, and each R_6 is independently hydrogen or a moiety that forms with the attached CO_2 group a readily hydrolyzable ester group. Preferably, R_6 is C_1-C_6 alkyl, hydroxyalkyl, alkylaryl or aralkyl. More preferably, R_6 is a C_1-C_2 alkyl.

The compound of the Formula XV is treated under conditions suitable to remove either R_4 or R_5, or both protecting groups, to obtain a compound of the Formula XVI:

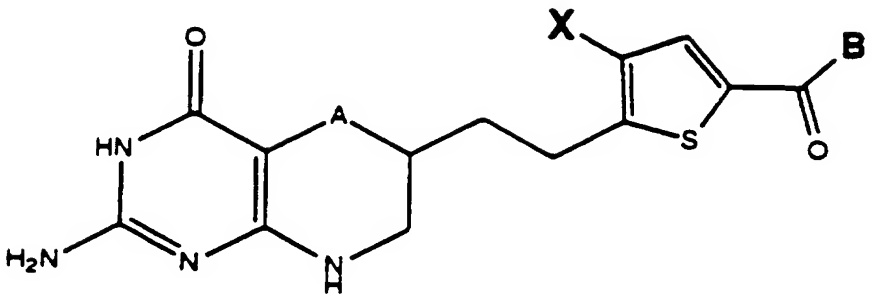


(XVI)

wherein A, X, B and R_6 are as defined above. Where t-butoxycarbonyl is used as a protecting group, suitable conditions are treatment with trifluoroacetic acid, followed by neutralization.

The compound of the Formula XVI is reacted with an alkylating agent, preferably trimethyl or triethyl oxonium tetrafluoroborate, in a suitable solvent, preferably dichloromethane, to form an intermediate lactim ether. The intermediate lactim ether is reacted with guanidine in an alcoholic solvent, preferably methanol,

ethanol or isopropanol, to form a compound of the Formula XVII:

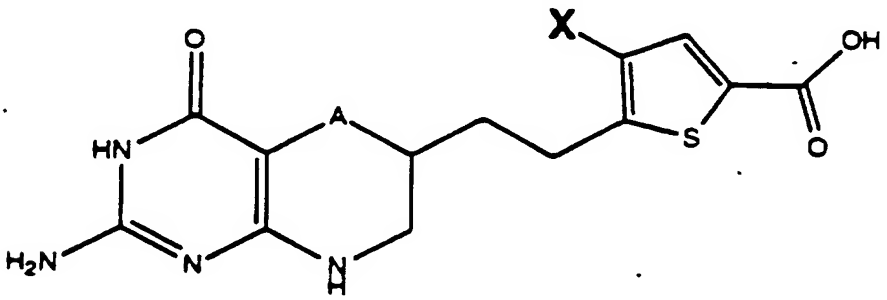


(XVII)

wherein A, X and B are as defined above.

Alternatively, the compound of the Formula XVI can be converted to the compound of the Formula XVII by reacting the compound of the Formula XVI with a thiolating agent, preferably P_2S_5 or 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide, to form the thiolactam intermediate. This intermediate is then alkylated with an alkylating agent, preferably methyl iodide or trimethyl or triethyl oxonium tetrafluoroborate, and then with guanidine in an alcoholic solvent, preferably methanol, ethanol or isopropanol, to obtain the compound of the Formula XVII.

Where B is an alcohol function--i.e., where the group attached with B forms an ester group--the compound of the Formula XVII is hydrolyzed under basic conditions to form a compound of the Formula XVIII:

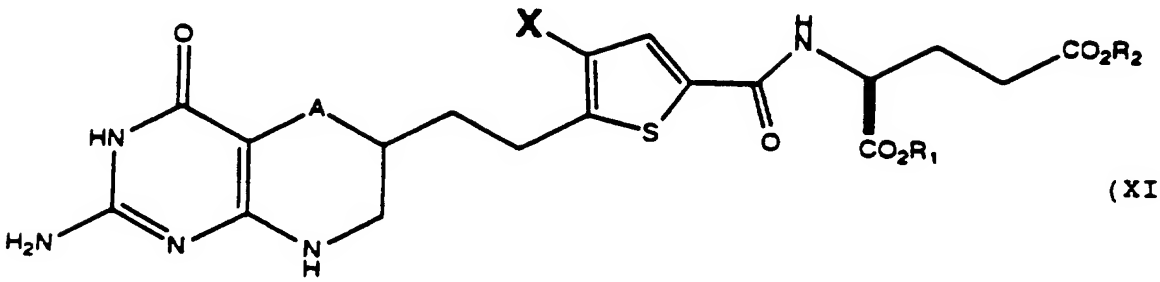


(XVIII)

wherein A and X are as defined above.

The compound of the Formula XVIII is peptide coupled, by means well known to those skilled in the art,

with a glutamic acid diester hydrochloride, to form a diester of the Formula XIX:

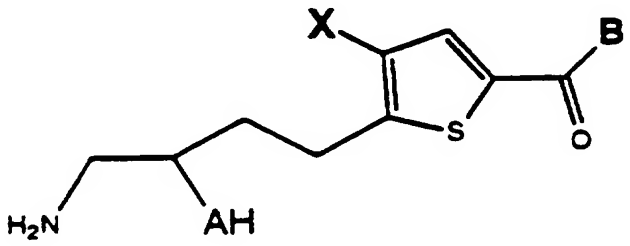


(XIX)

wherein A, X, R_1 and R_2 are as defined above, except that neither R_1 nor R_2 is hydrogen.

Finally, if the free glutamic acid form is desired, the compound of the Formula XIX is hydrolyzed to form a compound of the Formula I.

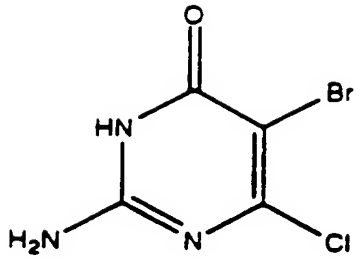
In the second conversion process, a compound of the Formula XIV is prepared as described above. This compound is treated with acid, preferably trifluoroacetic, hydrochloric or p-toluenesulfonic acid, to remove all of the protecting groups (R_4, R_5 and Ac) to obtain a compound of the Formula XX:



(XX)

wherein A, X and B are as defined above.

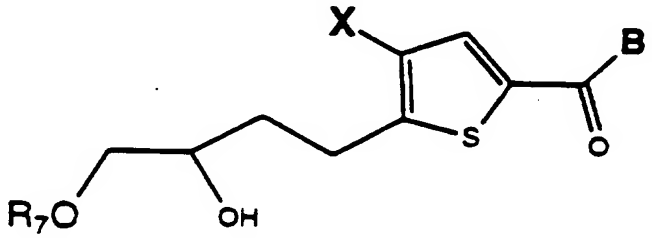
The compound of the Formula XX is reacted under weakly basic buffer conditions, preferably using a pH 7 phosphate buffer, in a suitable solvent, preferably ethanol or methanol, with a compound having the Formula XXI:



(XXI)

to obtain a compound of the Formula XVII. The remainder of the second process, proceeding from the compound of the Formula XVII to a compound of the Formula I, is conducted in a manner analogous to that described above.

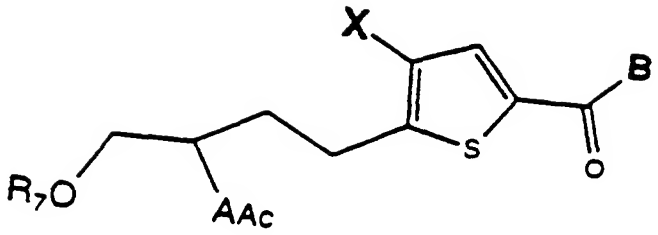
In the third conversion process, the compound of the Formula XI is reacted with a suitable hydroxyl-protecting group, preferably a trialkylsilyl group, more preferably a t-butyldimethylsilyl chloride, in the presence of a mild non-nucleophilic base, preferably triethylamine, to obtain a compound of the Formula XXII:



(XXII)

wherein X and B are as defined above, and R_7 is a suitable hydroxyl-protecting group, preferably a trialkylsilyl group.

The compound of the Formula XXII is then reacted with an acylating or sulfonylating agent, preferably methansulfonyl chloride or p-toluenesulfonyl chloride, in the presence of a non-nucleophilic base, preferably triethylamine or diisopropylethylamine, in a suitable solvent in which at least one of the reactants is at least partially soluble, to obtain an activated hydroxy group. The activated hydroxy group is displaced with a suitable nucleophile, preferably a thioacid salt, more preferably potassium thioacetate, to obtain a compound of the Formula XXIII:

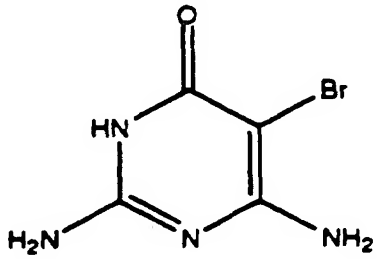


(XXIII)

wherein A, X, B, R_7 and Ac are as defined above.

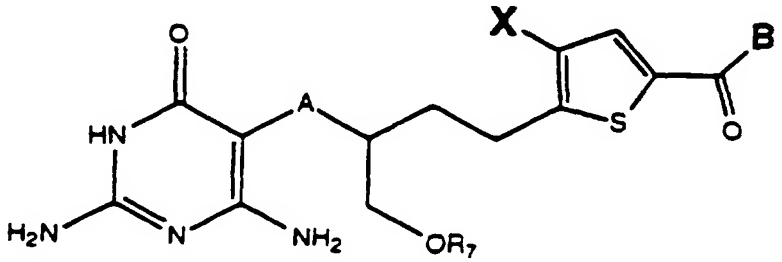
Alternatively, the compound of the Formula XXII can be converted to the compound of the Formula XXIII in one chemical operation using triphenylphosphine or diethyl or dimethyl azadicarboxylate, and an acidic nucleophile, preferably thioacetic acid, in a suitable solvent.

The compound of the Formula XXIII is reacted with a nucleophilic base or a mild acid to selectively remove the acyl group on moiety A. The resulting intermediate is reacted with a compound of the Formula XXIV:



(XXIV)

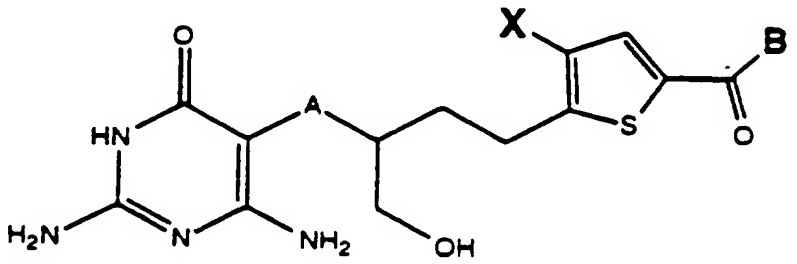
in the presence of a non-nucleophilic base, preferably triethylamine, diisopropylethylamine or potassium carbonate, to obtain a compound of the Formula XXV:



(XXV)

wherein A, X, B and R_7 are as defined above.

The protecting group R_7 on the compound of the Formula XXV is removed by treatment with a suitable reagent to obtain a compound of the Formula XXVI:

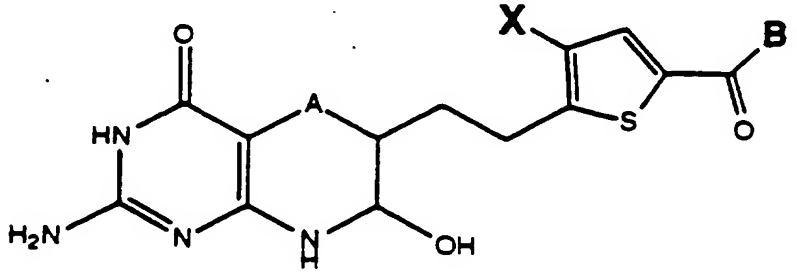


(XXVI)

wherein A, X and B are as defined above. Where R_7 is trialkylsilyl, the reagent is preferably a fluoride salt, more preferably potassium fluoride, tetrabutylammonium fluoride or cesium fluoride.

The compound of the Formula XXVI is cyclized to obtain the compound of the Formula XVII by activating the hydroxy group with an activating agent, preferably methanesulfonyl chloride, followed by treatment with a base. Alternatively, the nitrogen of the pyrimidinone is first protected with a suitable protecting group, preferably t-butoxycarbonyl, followed by cyclization and subsequent removal of the protecting group under acidic conditions. The remainder of the process proceeds from the compound of the Formula XVII to a compound of the Formula I in a manner analogous to that described above.

In the fourth and preferred conversion process, an alcohol compound of the Formula XXVI is prepared as described above. This alcohol is reacted with a suitable oxidizing agent to produce an aldehyde functionality that cyclizes to the compound of the Formula XXVII:

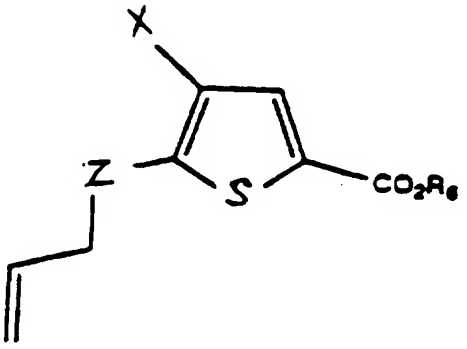


(XXVII)

wherein A, X and B are as defined above.

The compound of the Formula XXVII is reacted with a reducing agent, preferably sodium cyanoborohydride, in the presence of a Lewis acid, preferably boron trifluoride etherate, to obtain a compound of the Formula XVII defined above. The rest of the process proceeds from the compound of the Formula XVII to a compound of the Formula I in a manner analogous to that described above.

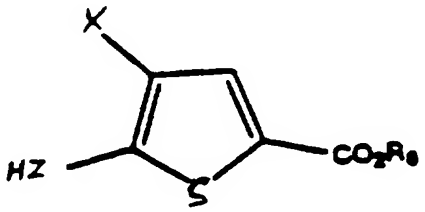
The compounds of the Formula I where Z is other than CH_2 can be prepared in an analogous manner to those where Z is CH_2. In particular, compounds of the Formula I wherein Z is other than CH_2 can be prepared using an olefin of the Formula XXXIV:



(XXXIV)

wherein X and R_6 are as defined above, and Z is as defined above for Formula I except that it is other than CH_2.

Where Z is sulfur, oxygen, or a substituted or unsubstituted amino, a compound of the Formula XXXV:

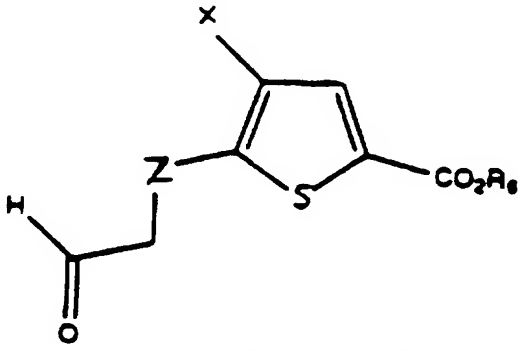


(XXXV)

wherein X and R_6 are as defined above, and Z is sulfur, oxygen, or a substituted or unsubstituted amino, is alkylated. The alkylation can be accomplished using an allylhalide, preferably allylbromide, in the presence of a

non-nucleophilic base, preferably triethylamine or diisopropylethylamine, to obtain the compound of the Formula XXXIV.

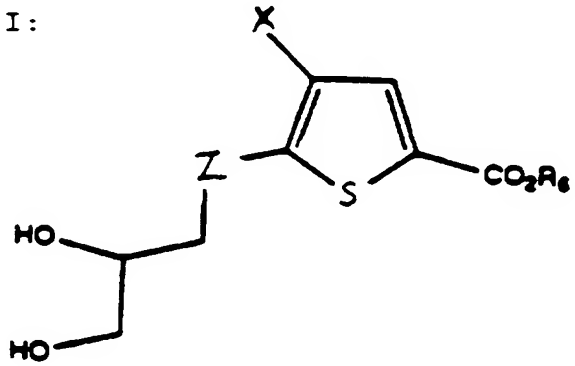
Where Z is a substituted or unsubstituted C_1-C_2 alkyl other than CH_2, a substituted or unsubstituted C_2-C_3 alkenyl or a substituted or unsubstituted C_2-C_3 alkynyl, the compound of the Formula XXXIV is prepared by olefination of an aldehyde of the Formula XXXVI:



(XXXVI)

wherein X and R_6 are as defined above, and Z is a substituted or unsubstituted C_1-C_2 alkyl other than CH_2, a substituted or unsubstituted C_2-C_3 alkenyl or a substituted or unsubstituted C_2-C_3 alkynyl. The aldehyde of the Formula XXXVI can be prepared in a manner analogous to that described by Chuan Shih et al., *Journal of Medicinal Chemistry*, vol. 35 (1992), 1109-1116. The olefination of the aldehyde can be accomplished using a methylene transfer agent, preferably methylene-triphenylphosphorane.

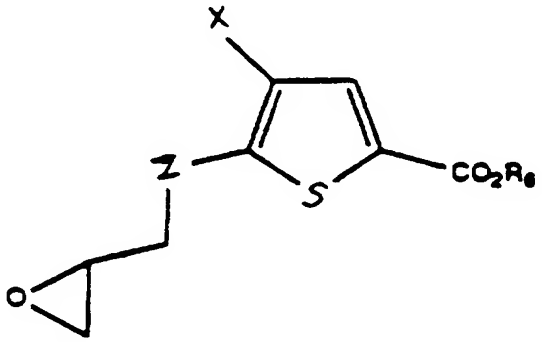
The compound of the Formula XXXIV is reacted with a dihydroxylating agent, preferably osmium tetroxide, in the presence of a suitable oxidizing agent, preferably N-methylmorpholine-N-oxide, to obtain a compound of the Formula XXXVII:



(XXXVII)

wherein X and R_6 are as defined above; and Z is as defined above for Formula I, except that it is other than CH_2.

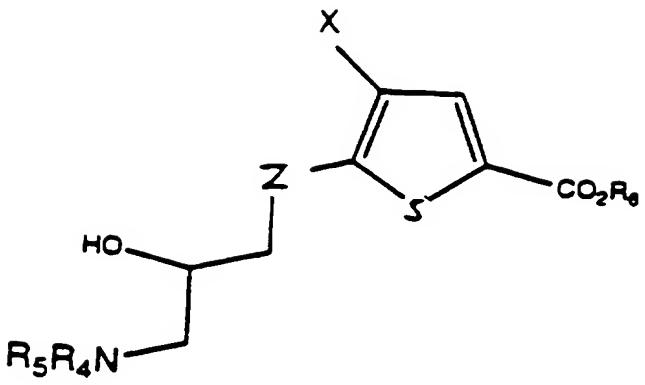
The compound of the Formula XXXVII is reacted with a sulfonylating agent, preferably p-toluenesulfonyl chloride or methanesulfonyl chloride, in the presence of a non-nucleophilic base, preferably triethylamine or diisopropylethylamine, to yield an intermediate mono-sulfonylated compound. This intermediate is reacted with a strong base, preferably sodium hydride, to produce a compound of the Formula XXXVIII:



(XXXVIII)

wherein X and R_6 are as defined above, and Z is as defined for Formula I except that it is other than CH_2.

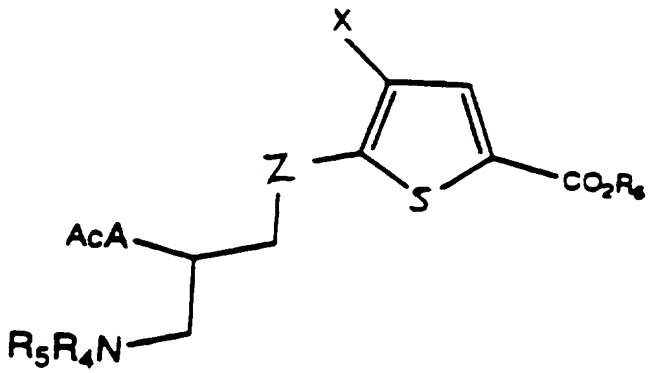
The epoxide of Formula XXXVIII is reacted with a nitrogen-containing nucleophile, preferably sodium azide, in the presence of a mild Lewis-acid catalyst, preferably lithium or magnesium perchlorate, to an obtain an intermediate alcohol azide. This intermediate is reduced, preferably with hydrogen gas in the presence of a metal catalyst, and subsequent protection with a suitable nitrogen-protecting group, preferably t-butoxycarbonyl, benzoxycarbonyl or benzyl, to produce a compound of the Formula XVII':



(XVII')

wherein X, R_6, and R_4 and R_5 are as defined above, and Z is as defined for Formula I except that it is other than CH_2.

The compound of the Formula XVII' is then reacted with an acylating or sulfonylating agent, preferably methanesulfonyl chloride or p-toluenesulfonyl chloride, in the presence of a non-nucleophilic base, preferably triethylamine or diisopropylethylamine, in a suitable solvent in which at least one of the reactants is at least partially soluble, to obtain an activated hydroxy group. The activated hydroxy group is displaced with a suitable nucleophile, preferably a thioacid salt, more preferably potassium thioacetate, to obtain a compound of the Formula XVIII':

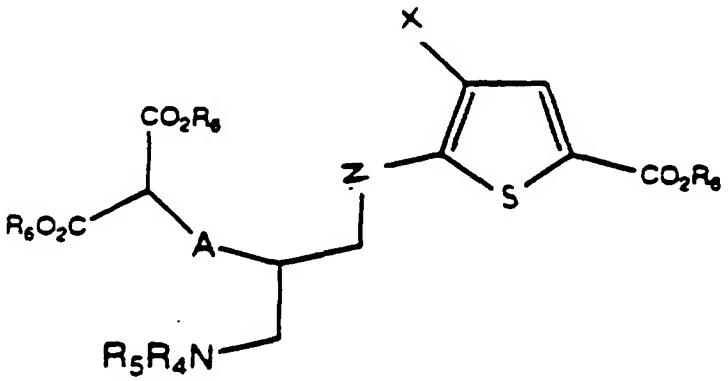


(XVIII')

wherein A, X, R_6, R_4 and R_5, and Ac are as defined above, and Z is as defined for Formula I except that it is other than CH_2.

Alternatively, the compound of Formula XVII' is converted to the compound of Formula XVIII' in one chemical operation using triphenylphosphine, diethyl or dimethyl aza-dicarboxylate, and an acidic nucleophile, preferably thioacetic acid, in a suitable solvent.

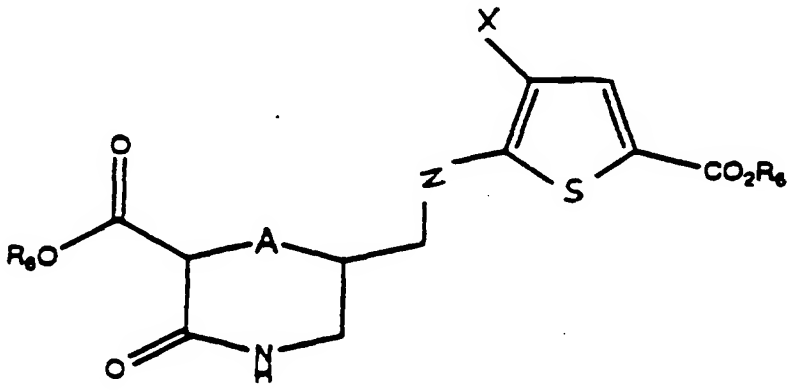
The compound of the Formula XVIII' is treated with a nucleophilic base, preferably potassium carbonate, sodium carbonate, sodium hydroxide or potassium hydroxide, in an alcoholic solvent, preferably methanol, ethanol or isopropanol, in the presence of an alkylating agent, preferably dimethyl or diethyl chloromalonate, to obtain a compound of the Formula XIX':



(XIX')

wherein A, X, R_6, and R_4 and R_5 are as defined above, and Z is as defined for Formula I except that it is other than CH_2.

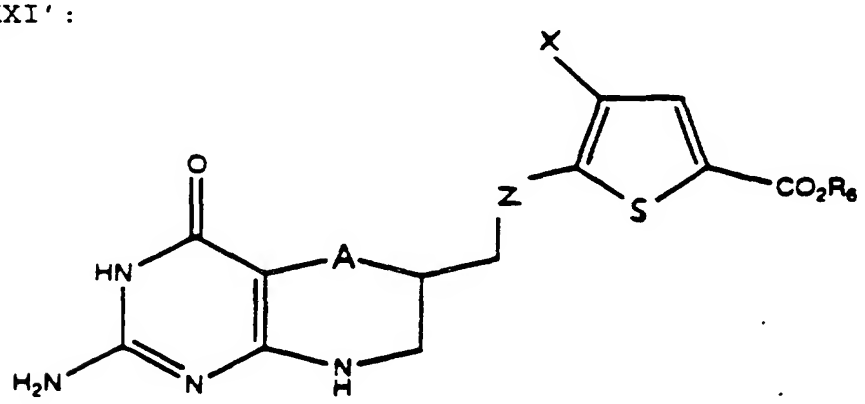
The compound of the Formula XIX' is treated under conditions suitable to remove either or both of the R_4 and R_5 protecting groups to produce a compound of the Formula XX':



(XX')

wherein A, X and R_6 are as defined above, and Z is as defined for Formula I except that it is other than CH_2. Where t-butoxycarbonyl is a protecting group, the conditions for removal of this group are preferably treatment with trifluoroacetic acid followed by neutralization to produce the compound of the Formula XX'.

The compound of the Formula XX' is reacted with an alkylating agent, preferably trimethyl or triethyl oxonium tetrafluoroborate, in a suitable solvent, preferably dichloromethane, to form an intermediate lactim ether. The intermediate lactim ether is reacted with guanidine in an alcoholic solvent, preferably methanol, ethanol or isopropanol, to form a compound of the Formula XXI':

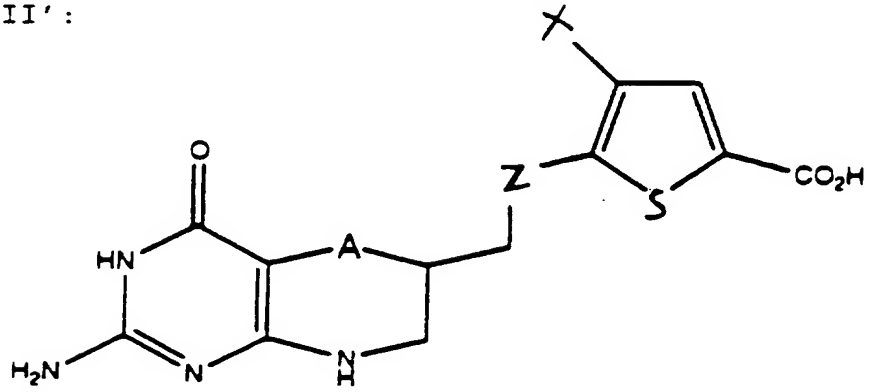


(XXI')

wherein A, X and R_6 are as defined above, and Z is as defined for Formula I except that it is other than CH_2.

Alternatively, the compound of the Formula XX' is converted to the compound of the Formula XXI' by reacting the compound of the Formula X' with a thiolating agent, preferably P_2S_5 or 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide to form the thiolactam intermediate. This can then be alkylated with an alkylating agent, preferably methyl iodide or trimethyl or triethyl oxonium tetrafluoroborate, and then with guanidine in an alcoholic solvent, preferably methanol, ethanol or isopropanol, to obtain the compound of the Formula XXI'.

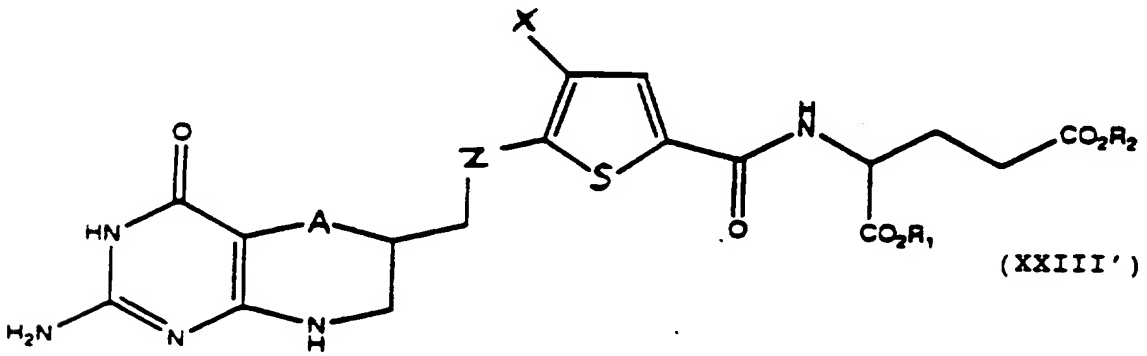
The compound of the Formula XXI' is hydrolyzed under basic conditions to form a compound of the Formula XXII':



(XXII')

wherein A and X are as defined above, and Z is as defined for Formula I except that it is other than CH_2. Where R_6 is hydrogen in the compound of the Formula XXI', then the hydrolyzation reaction is not necessary, and the compound of the Formula XXI' is peptide coupled as described below.

The compound of the Formula XXII' (or the compound of the Formula XXI' where R_6 is hydrogen), which is in the free carboxylic acid form, can be peptide coupled, by means well known to those skilled in the art, with a glutamic acid diester hydrochloride to form a diester of the Formula XXIII':



(XXIII')

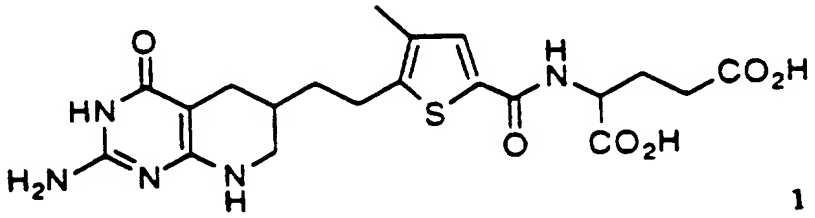
wherein A, X and are as defined for Formula XXII', and R_1 and R_2 are each independently a moiety that forms with the attached CO_2 a readily hydrolyzable ester group, such as a C_1-C_6 alkyl, hydroxyalkyl, alkylaryl or arylalkyl.

Finally, if the free acid form is desired, the compound of the Formula XXIII' is hydrolyzed to produce compounds of the Formula I where R_1 and R_2 are each H.

A detailed example of the preparation of a compound of the Formula I is provided below.

EXAMPLE 1

N-(5-[2-(2-amino-4(3H)-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]-4-methylthieno-2-yl)-L-glutamic acid (Compound 1)

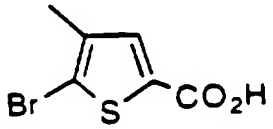


1

Synthesis

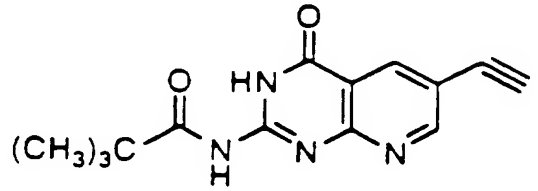
Compound 1 was synthesized by the following process.

a. 5-bromo-4-methylthiophene-2-carboxylic acid:



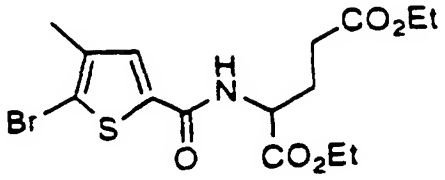
This compound was prepared according to M. Nemec, *Collection Czechoslov. Chem. Commun.*, vol. 39 (1974), 3527.

b. 6-ethynyl-2-(pivaloylamino)-4(3H)-oxopyrido [2,3-d] pyrimidine:



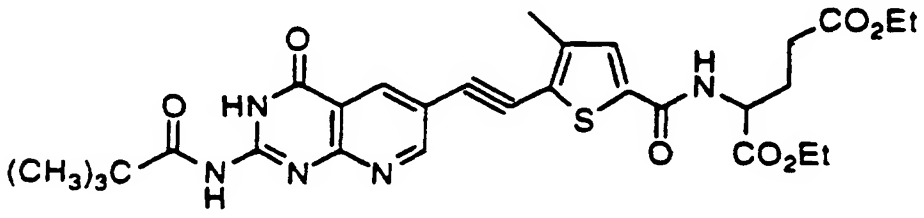
This compound was prepared according to E.C. Taylor & G.S.K. Wong, *J. Org. Chem.*, vol. 54 (1989), 3618.

c. Diethyl N-(5-bromo-4-methylthieno-2-yl)-L-glutamate:



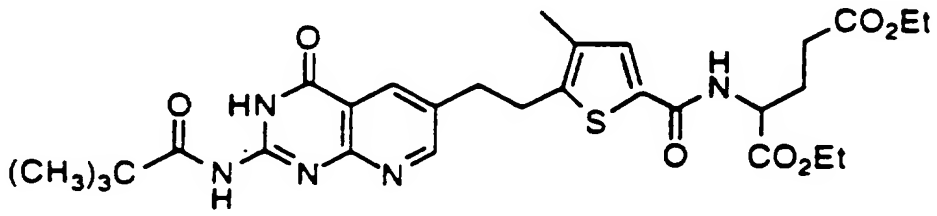
To a stirred solution of 5-bromo-4-methyl-thiophene-2-carboxylic acid (3.32 g, 15 mmol), 1-hydroxybenzotriazole (2.24 g, 16.6 mmol), L-glutamic acid diethyl ester hydrochloride (3.98 g, 16.6 mmol) and diisopropylethylamine (2.9 ml, 2.15 g, 16.6 mmol) in dimethylformamide (DMF) (40 ml) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.18 g, 16.6 mmol). The resulting solution was stirred under argon at ambient temperature for 18 hours, poured into brine (300 ml), diluted with water (100 ml) and extracted with ether (3 x 120 ml). The combined organic extracts were washed with water (150 ml), dried over $MgSO_4$ and concentrated *in vacuo* to give a brown gum, which was purified by flash chromatography. Elution with hexane: EtOAc (2:1) provided the product as an orange oil (5.05 g, 83% yield). Analyses indicated that the product was diethyl N-(5-bromo-4-methylthieno-2-yl) glutamate. NMR($CDCl_3$) δ: 7.22 (1H, s), 6.86 (1H, d, J = 7.5 Hz), 4.69 (1H, ddd, J = 4.8, 7.5, 9.4 Hz), 4.23 (2H, q, J = 7.1 Hz), 4.12 (2H, q, J = 7.1 Hz), 2.55 - 2.39 (2H, m), 2.35 - 2.22 (1H, m), 2.19 (3H, s), 2.17 - 2.04 (1H, m), 1.29 (3H, t, J = 7.1 Hz), 1.23 (3H, t, J = 7.1 Hz). Anal. ($C_{15}H_{20}NO_5SBr$) C,H,N,S,Br.

d. diethyl N-(5-[(2-[pivaloylamino]-4(3H)-oxopyrido [2,3-d] pyrimidin-6-yl) ethynyl]-4-methylthieno-2-yl) glutamate:



To a stirred solution of diethyl N-(5-bromo-4-methylthieno-2-yl) glutamate (4.21 g, 10.4 mmol) in acetonitrile (55 ml) under an argon atmosphere were added bis (triphenyl-phosphine) palladium chloride (702 mg, 1.0 mmol), cuprous iodide (200 mg, 1.1 mmol), triethylamine (1.5 ml, 1.09 g, 10.8 mmol) and 6-ethynyl-2-(pivaloylamino)-4(3H)-oxopyrido[2,3-d]pyrimidine (5.68 g, 21 mmol). The resultant suspension was heated at reflux for 6 hours. After cooling to room temperature, the crude reaction mixture was filtered and the precipitate was washed with acetonitrile (50 ml) and ethylacetate (EtOAc) (2 x 50 ml). The combined filtrates were concentrated *in vacuo* to give a brown resin, which was purified by flash chromatography. Elution with CH_2Cl_2:CH_3OH (49:1) provided the product as an orange solid (4.16 g, 67% yield). Analyses indicated that the product was diethyl N-(5-[(2-[pivaloylamino]-4(3H)-oxopyrido[2,3-d]pyrimidin-6-yl) ethynyl]-4-methylthieno-2-yl) glutamate. NMR ($CDCl_3$) δ: 8.95 (1H, d, J = 2.2 Hz), 8.59 (1H, d, J = 2.2 Hz), 7.33 (1H, s), 7.03 (1H, d, J = 7.4 Hz), 4.73 (1H, ddd, J = 4.8, 7.4, 9.5 Hz), 4.24 (2H, q, J = 7.1 Hz), 4.13 (2H, q, J = 7.1 Hz), 2.55 - 2.41 (2H, m), 2.38 (3H, s), 2.35 - 2.24 (1H, m), 2.19 - 2.05 (1H, m), 1.34 (9H, s), 1.30 (3H, t, J = 7.1 Hz), 1.24 (3H, t, J = 7.1 Hz). Anal. ($C_{29}H_{33}N_5O_7S.0.75H_2O$) C,H,N,S.

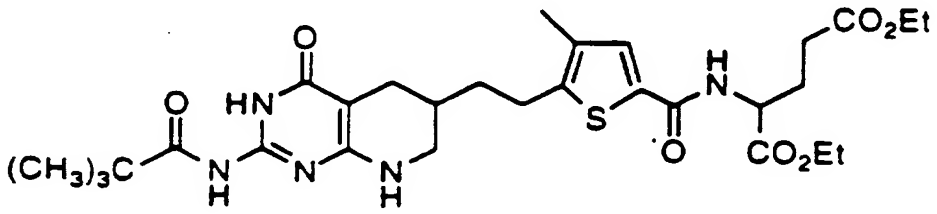
e. diethyl N-(5-[(2-[pivaloylamino]-4(3H)-oxopyrido [2,3,d] pyrimidin-6-yl)ethyl]-4-methylthieno-2-yl) glutamate:



A suspension of diethyl N-(5-[(2-[pivaloylamino]-4(3H)-oxopyrido[2,3-d]pyrimidin-6-yl)ethyl]-4-methylthieno-2-yl) glutamate (959 mg, 1.6 mmol) and 10% Pd on carbon (1.5 g,

150 % wt. eq.) in trifluoroacetic acid (30 ml) was shaken under 50 psi of H_2 for 22 hours. The crude reaction mixture was diluted with CH_2Cl_2, filtered through a pad of Celite (diatomaceous earth) and concentrated *in vacuo*. The residue obtained was dissolved in CH_2Cl_2 (120 ml), washed with saturated $NaHCO_3$ (2 x 100 ml), dried over Na_2SO_4 and concentrated *in vacuo* to give a brown gum, which was purified by flash chromatography. Elution with CH_2Cl_2:CH_3OH (49:1) provided the product as a yellow solid (772 mg, 80% yield). Analyses indicated that the product was diethyl N-(5-[(2-[pivaloylamino]-4(3H)-oxopyrido[2,3-d]pyrimidin-6-yl)ethyl]-4-methylthieno-2-yl) glutamate. NMR ($CDCl_3$) δ: 8.60 (1H, d, J = 2.2 Hz), 8.49 (1H, broad), 8.32 (1H, d, J = 2.2 Hz), 7.22 (1H, s), 6.78 (1H, d, J = 7.5 Hz), 4.72 (1H, ddd, J = 4.8, 7.5, 9.5 Hz), 4.23 (2H, q, J = 7.1 Hz), 4.11 (2H, q, J = 7.1 Hz), 3.12 - 3.00 (4H, m), 2.52 - 2.41 (2H, m), 2.37 - 2.22 (1H, m), 2.16 - 2.04 (1H, m), 2.02 (3H, s), 1.33 (9H, s), 1.29 (3H, t, J = 7.1 Hz), 1.23 (3H, t, J = 7.1 Hz). Anal. ($C_{29}H_{37}N_5O_7S.0.5H_2O$) C,H,N,S.

f. diethyl N-(5-[(2-[pivaloylamino]-4(3H)-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)-ethyl]-4-methylthieno-2-yl) glutamate:



A suspension of diethyl N-(5-[(2-[pivaloylamino]-4(3H)-oxopyrido[2,3-d]pyrimidin-6-yl)ethyl]-4-methylthieno-2-yl) glutamate (2.98 g, 5 mmol), 10% Pt on carbon (1.5 g, 50% wt. eq.) and PtO_2 (1.5 g, 50% wt. eq.) in trifluoroacetic acid (170 ml) was shaken under 800 psi of H_2 for 40 hours. The crude reaction mixture was diluted with CH_2Cl_2, filtered through a pad of Celite, and concentrated *in vacuo*. The residue obtained was dissolved in CH_2Cl_2 (150 ml), washed with saturated $NaHCO_3$ (2 x 150 ml), dried over

Na_2SO_4, and concentrated *in vacuo* to give a brown resin, which was purified by flash chromatography. Elution with CH_2Cl_2:CH_3OH (24:1) provided initially an unreacted substrate (1.42 g, 48% yield) and then the product as a yellow solid (293 mg, 10% yield). Analyses indicated that the product was diethyl N-(5-[(2-[pivaloylamino]-4(3H)-oxo-5,6,7,8-tetrahydropyrido-[2,3-d]pyrimidin-6-yl)ethyl]-4-methylthieno-2-yl) glutamate. NMR ($CDCl_3$) δ: 7.24 (1H, s), 6.75 (1H, d, J = 7.6 Hz), 5.57 (1H, broad), 4.72 (1H, ddd, J = 4.8, 7.6, 12.6 Hz), 4.22 (2H, q, J = 7.1 Hz), 4.11 (2H, q, J = 7.1 Hz), 3.43 - 3.36 (1H, m), 3.06 - 2.98 (1H, m), 2.89 - 2.68 (3H, m), 2.52 - 2.40 (3H, m), 2.37 - 2.23 (1H, m), 2.15 (3H, s), 2.14 - 2.03 (1H, m), 1.94 - 1.83 (1H, m), 1.73 - 1.63 (2H, m), 1.32 (9H,s), 1.29 (3H, t, J = 7.1 Hz), 1.23 (3H, t, J = 7.1 Hz). Anal. ($C_{29}H_{41}N_5O_7S.0.5H_2O$) C,H,N,S.

g. N-(5-[2-(2-amino-4(3H)-oxo-5,6,7,8-tetrahydropyrido- [2,3-d]pyrimidin-6-yl)ethyl]-4-methylthieno-2-yl) glutamic acid (Compound **1**):

A solution of diethyl N-(5-[(2-[pivaloylamino]-4(3H)-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]-4-methylthieno-2-yl) glutamate (293 mg, 0.5 mmol) in 1N NaOH (25 ml) was stirred at ambient temperature for 90 hours, then neutralized with 6N HCl. The precipitate that formed was collected by filtration and washed with water (4 x 10 ml) to provide the product as a yellow solid (63 mg, 28% yield). Analyses indicated that the product was N-(5-[2-(2-amino-4(3H)-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]-4-methylthieno-2-yl) glutamic acid. NMR (DMSO-d6) δ: 12.44 (2H, broad), 9.89 (1H, broad), 8.42 (1H, d, J = 7.8 Hz), 7.57 (1H, s), 6.39 (1H, br s), 6.12 (2H, br s), 4.30 (1H, ddd, J = 4.8, 7.8, 9.6 Hz), 3.26 - 3.18 (2H, m), 2.83 - 2.74 (3H, m), 2.31 (2H, t, J = 7.4 Hz), 2.12 (3H, s), 2.09 - 2.01 (1H, m), 1.94 - 1.80 (2H, m), 1.68 - 1.47 (3H,m). Anal. ($C_{20}H_{25}N_5O_6S.1.1H_2O$) C,H,N,S.

Biological and Biochemical Evaluation

Determination of Inhibition Constants for GAR Transformylase:

The GAR-transformylase (GARFT) assay method of Young et al., *Biochemistry* 23 (1984), 3979-3986, was modified and used as described below. Reactions mixtures contained the catalytic domain of the human GARFT, 0-250 nM of the test compound, 20 μM glycinamide ribonucleotide (GAR), 10 or 20 μM N^{10}-formyl-5,8-dideazafolate (FDDF), 50 mM HEPES-KOH (pH 7.5), and 50 mM KCl. The reaction was initiated with the addition of enzyme to a final concentration of 11 nM, followed by monitoring of the increase in absorbance at 294 nm at 20°C (e_{294} = 18.9 mM^{-1} cm^{-1}).

The GARFT inhibition constant (K_i) was determined from the dependence of the steady-state catalytic rate on inhibitor and substrate concentration. The type of inhibition observed was determined to be competitive with respect to FDDF by the dependence of the apparent K_i ($K_{i,app}$) on the concentration of FDDF and was shown to be described by $K_{i,app} = K_i + (K_i/K_m)[FDDF]$.. The Michaelis constant for FDDF, K_m, was determined independently by the dependence of the catalytic rate on FDDF concentration. Data for both the K_m and K_i determinations were fitted by non-linear methods to the Michaelis equation, or to the Michaelis equation for competitive inhibition, as appropriate. Data resulting from tight-binding inhibition was analyzed and K_i was determined by fitting the data to the tight-binding equation of Morrison, *Biochem Biophys Acta* 185 (1969), 269-286, by nonlinear methods.

Determination of Dissociation Constants for Human Folate Binding Protein:

The dissociation constant (Kd) for human folate-binding protein (FBP) was determined in a competitive binding assay using membrane associated FBP prepared from cultured KB cells.

Preparation of KB cell Membrane Fraction:

Adherent KB cells were scraped from flasks, washed once in ice-cold PBS, and centrifuged at 5000 x g for 5 minutes at 4°C. Pelleted cells ($2x10^8$ cells) were resuspended in 10 ml of suspension buffer (KH_2PO_4-KOH pH 7.4 : 10 mM EDTA : 10 mM 2-mercaptoethanol), sonicated briefly to complete cell lysis and centrifuged at 12000 x g for 10 minutes at 4°C. The pellet was stripped of endogenous bound folate by resuspension in 20 ml of acidic buffer (50 mM KH_2PO_4-KOH pH 3.5 : 10 mM EDTA : 10 mM 2-mercaptoethanol) and centrifuged as before. The pellet was then resuspended in 20 ml of the suspension buffer at pH 7.4 and centrifuged as before. The pellet was resuspended in 5 ml of suspension buffer at pH 7.4 lacking EDTA. Protein content was quantitated using the Bradford method with BSA as standard. Typical yields for this procedure were 4-5 mg total membrane protein per $2x10^8$ cells. This final suspension was used as a source of membrane-associated human FBP.

FBP Competitive Binding Assay:

Inhibitor was allowed to compete against ^{3}H-folic acid for binding to FBP. Reactions mixtures contained 50-100 mg of cell membrane protein containing 3-6 pmoles (3-6 nM) of FBP, 17.25 pmoles ^{3}H-folic acid (17.25 nM, 0.5 μCi), various concentrations of competitor, in 1 ml of 50 mM KH_2PO_4-KOH pH 7.4 : 10 mM 2-mercaptoethanol. Reactions were performed at 25°C. Because of the very slow release of bound ^{3}H-folic acid, the competitor was prebound for 30 minutes in the absence of ^{3}H-folic acid. ^{3}H-Folic acid was then added and the mixtures were allowed to equilibrate for 2.5 hours. The full reaction mixtures were drawn through nitrocellulose filters under vacuum to trap the cell membranes with bound ^{3}H-folic acid. The trapped membranes were then washed 4 times with 1 ml of reaction buffer. The amount of bound ^{3}H-folic acid was measured by scintillation counting of the nitrocellulose membrane. The data obtained were nonlinearly fitted as described above. The FBP K_d for ^{3}H-folic acid, used to calculate the competitor K_d, was

obtained by direct titration of FBP with ^{3}H-folate and subsequent nonlinear fitting of the data to a tight-binding K_d equation.

Cell lines:

The cell lines used and their origin are tabulated in Table 1. The growth conditions and media requirements of each cell line are summarized in Table 2. All cultures were maintained at 37°C, 5% air-CO_2 in a humidified incubator.

In vitro growth inhibition:

Stock solutions of the inhibitors were prepared in 10 mM sodium bicarbonate in water and stored in 1 ml aliquots at -20°C for cell culture experiments. Cell-growth inhibition was measured by a modification of the method of Mosmann, *J. Immunol. Methods* 65 (1983), 55-63.

Mid-log phase cells of each cell line were diluted to 18,500 cells/ml in fresh RPMI growth medium (Mediatech, Washington, DC) supplemented with dialyzed fetal-calf serum (Hyclone Laboratories Inc., Logan, UT), and then aliquotted into columns 2 through 12 of 96-well microtiter plates. Column 1 was filled with the same volume, 135 ml, of fresh medium, without cells, for use as a blank. The plates were then placed in a 37°C, 5% air-CO_2 incubator. After 1 to 4 hours, plates were removed from the incubator followed by addition of the test compound at 10 x final concentration, 15 ml/well in binary dilutions, to columns 12 to 4. For reversal experiments, hypoxanthine (1.75 mM) or AICA (1.75 mM) was included in all drug solutions (final concentration 175 mM). Wells containing each concentration of test compound were prepared in quadruplicate on each plate. Fifteen milliliters of media, without test compound, were added to the wells in column 1 of the plates. The cells were then returned to the incubator and remained undisturbed for the full incubation period. On day 3 for L1210 and L1210/CI920 cells or day 5 for CCRF-CEM cells, 50 ml of 0.8 mg/ml MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium

bromide; Sigma catalog no. M2128) dissolved in tissue culture medium was added to each well of all plates, after which cells were returned to the incubator. After 4 hours, all plates were removed from the incubator and centrifuged at 1200 rpm for 7 minutes. Media were siphoned off and 150 ml of DMSO was added to each well of all plates. Plates were then mixed at slow speed on a vortex mixer for 1 hour in the dark at room temperature. The extent of metabolized MTT was measured spectrophotometrically at 540 nm on a Molecular Devices Vmax™ kinetic microplate reader. The concentration of drug required to reduce cell growth by 50% as measured by MTT metabolism was determined by interpolation between the O.D. (minus blank) immediately above and below 50% of control O.D. (minus blank).

TABLE 1

Tissue of Origin and Source of Cell Lines Employed in *In Vitro* Studies

Cell Line	Source	Origin
L1210	ATCC#	Mouse, lymphocytic leukemia
CCRF-CEM	ATCC#	Human, acute lymphoblastic leukemia

#ATCC = American Type Culture Collection

TABLE 2

Culture Conditions, Plating Densities and Incubation Times Used in Microtiter Assays

Cell line	Medium	DFCS Conc.* (%)	Plating Density (cells/well)	Incubation Time (days)
L1210	RPMI-1640	5	2500	3
CCRF-CEM	RPMI-1640	10	2500	5

*DFCS Conc. = dialyzed fetal calf serum concentration.

TABLE 3

Comparative Data for Test Compound and 6R-DDATHF Growth Inhibition Using Continuous (72-hour) Exposure

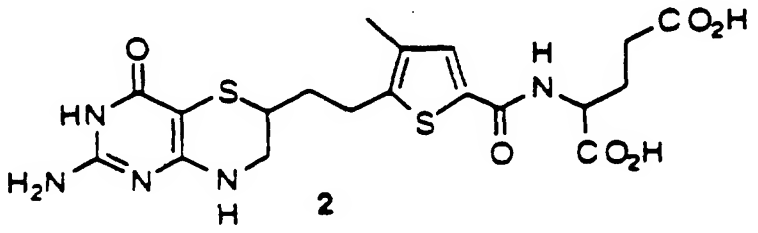
Compound	GARFT K_i (nM)	IC_{50} Cell Culture L1210 (nM)[a]	IC_{50} Cell Culture CCRF-CEM (nM)[a]	Human Folate Binding Protein K_d (nM)
1	1.4	13.5	6.1	28
DDATHF[b]	25	17.5	1.5	0.020

a: Mean IC_{50} ± standard deviation;
b: 6R-DDATHF, the 6R diastereomer of 5,,10-dideazatetrahydrofolic acid (Lometrexol) (See F.M. Muggia, "Folate antimetabolites inhibitor to *de novo* purine synthesis," *New Drugs, Concepts and Results in Cancer Chemotherapy*, Kluwer Academic Publishers, Boston (1992), 65 87.

As the above comparative data show, Compound **1** has a relative folate binding protein K_d that is about 1400 times less potent than 6R-DDATHF.

EXAMPLE 2

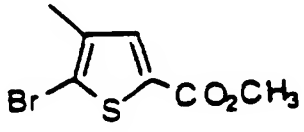
N-(5-[2-(2-amino-4-oxo-4,6,7,8-tetrahydro-3H-pyrimido[5,4-6]- [1,4]thiazin-6-yl)ethyl]-4-methylthieno-2-yl)-L-glutamic acid (Compound 2)



2

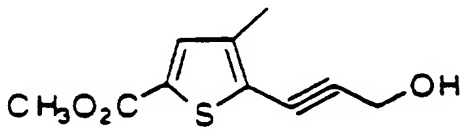
Compound **2** was prepared as follows.

a. methyl 5-bromo-4-methylthiophene-2-carboxylate:



To a solution of 5-bromo-4-methylthiophene-2-carboxylic acid (20.32 g, 92 mmol) in CH_3OH (450 ml) was added concentrated H_2SO_4 (4 ml). The resultant solution was heated at reflux for 18 hours. The solvent was removed by concentration *in vacuo*, and the residue obtained was partitioned between saturated $NaHCO_3$ (350 ml) and ether (350 ml). The layers were separated and the aqueous phase extracted with ether (3 x 150 ml). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo* to give a red oil, which was purified by flash chromatography. Elution with hexane:ethyl acetate (9:1) provided the product as a yellow oil, which solidified on standing (18.34 g, 85% yield). Analyses indicated that the product was methyl 5-bromo-4-methyl-thiophene-2-carboxylate. NMR ($CDCl_3$) δ: 7.47 (1H, s), 3.86 (3H, s), 2.20 (3H, s). Anal. ($C_7H_7O_2SBr$) C,H,S,Br.

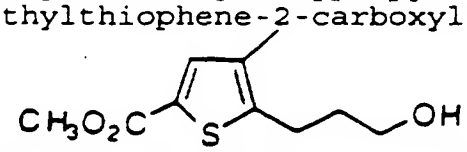
b. methyl 5-(3-hydroxypropynyl)-4-methylthiophene-2-carboxylate:



To a stirred solution of methyl 5-bromo-4-methyl-thiophene-2-carboxylate (5.18 g, 22 mmol) in diethylamine (60 ml) under an argon atmosphere were added bis(triphenylphosphine) palladium chloride (77 mg, 0.11 mmol), cuprous iodide (42 mg, 0.22 mmol) and propargyl alcohol (1.5 ml, 1.44 g, 26 mmol). The resultant mixture was stirred at ambient temperature for 18 hours. The solvent was removed by concentration *in vacuo*, and the residue obtained was diluted with water (200 ml) and then extracted with EtOAc (3 x 100 ml). The combined organic extracts were washed with 0.5 N HCl (100 ml), dried over $MgSO_4$ and concentrated *in vacuo* to give a brown oil, which was purified by flash chromatography. Elution with hexane:EtOAc (2:1) provided the product as an orange oil,

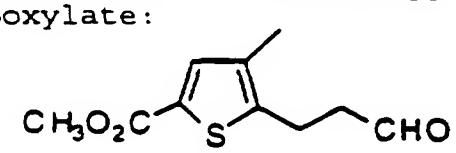
which solidified on standing (4.07 g, 88% yield). Analyses indicated that the product was methyl 5-(3-hydroxypropynyl)-4-methylthiophene-2-carboxylate. NMR ($CDCl_3$) δ: 7.52 (1H, s), 4.55 (2H, s), 3.87 (3H, s), 2.29 (3H, s). Anal. ($C_{10}H_{10}O_3S$) C,H,S.

c. methyl 5-(3-hydroxypropyl)-4-methylthiophene-2-carboxylate:



A suspension of methyl 5-(3-hydroxypropynyl)-4-methyl-thiophene-2-carboxylate (3.86 g, 18 mmol) and 5% Pd on carbon (0.72 g, 19% wt. eq.) in EtOAc (110 ml) was shaken under 50 psi of H_2 for 20 hours. The crude reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to provide the product as a yellow oil (3.84 g, 98% yield). Analyses indicated that the product was methyl 5-(3-hydroxypropyl)-4-methylthiophene-2-carboxylate. NMR ($CDCl_5$) δ: 7.51 (1H, s), 3.84 (3H, s), 3.71 (2H, t, J = 6.2 Hz), 2.86 (2H, t, J = 7.6 Hz), 2.16 (3H, s), 1.92 (2H, tt, J = 6.2, 7.6 Hz). Anal. ($C_{10}H_{14}O_3S$) C,H,S.

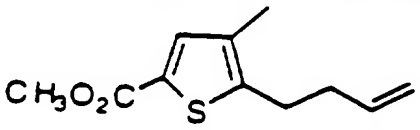
d. methyl 4-methyl-5-(3-oxopropyl) thiophene-2-carboxylate:



To a stirred suspension of methyl 5-(3-hydroxypropyl)-4-methylthiophene-2-carboxylate (3.74 g, 17 mmol), N-methylmorpholine-N-oxide (3.00 g, 26 mmol) and powdered 4Å molecular sieves (4.5 g) in CH_2Cl_2 (50 ml) was added tetrapropylammonium perruthenate (300 mg, 0.85 mmol). The resultant suspension was stirred at ambient temperature for 40 minutes. The solvent was removed by concentration *in vacuo*, and the residue obtained was purified by flash chromatography. Elution with hexane:EtOAc (4:1) provided the product as a yellow oil (1.82 g, 49% yield). Analyses

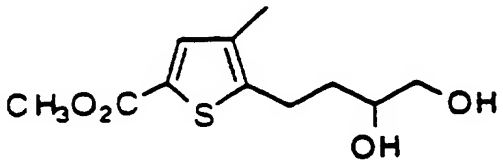
indicated that the product was methyl 4-methyl-5-(3-oxopropyl) thiophene-2-carboxylate. NMR ($CDCl_3$) δ: 9.83 (1H, t, J = 0.8 Hz), 7.50 (1H, s), 3.84 (3H, s), 3.07 (2H, t, J = 7.4 Hz), 2.83 (2H, dt, J = 0.8, 7.4 Hz), 2.17 (3H, s). Anal. ($C_{10}H_{12}O_3S$) C,H,S

e. ethyl 5-(3-butenyl)-4-methylthiophene-2-carboxylate:



To a stirred suspension of methyltriphenylphosphonium bromide (3.14 g, 8.8 mmol) in THF (30 ml) under an argon atmosphere at 0°C was added 2.5 M n-butyllithium in hexane (3.4 ml, 8.5 mmol). The resultant slurry was stirred for 10 minutes at 0°C, for 75 minutes at ambient temperature, and then cooled to -65°C prior to the dropwise addition of a solution of the methyl 4-methyl-5-(3-oxopropyl) thiophene-2-carboxylate (1.71 g, 8.1 mmol) in THF (30 ml). The cooling bath was removed and the reaction was stirred for 90 minutes while gradually warming to room temperature. The crude reaction mixture was concentrated *in vacuo* to a volume of 20 ml, diluted with ether (200 ml), and filtered through a pad of celite. The filtrate was concentrated *in vacuo* to give an orange oil, which was purified by flash chromatography. Elution with hexane:EtOAc (95:5) provided the product as a yellow oil (772 mg, 46%). Analyses indicated that the product was methyl 5-(3-butenyl)-4-methylthiophene-2-carboxylate. NMR ($CDCl_3$) δ: 7.50 (1H, s), 5.84 (1H, ddt, J = 10.2, 17.0, 6.6 Hz), 5.07 (1H, dd, J = 1.6, 17.0 Hz), 5.02 (1H, dd, J = 1.6, 10.2 Hz), 3.84 (3H, s). Anal. ($C_{11}H_{14}O_2S$) C,H,S.

f. methyl 5-(3,4-dihydroxybutyl)-4-methylthiophene-2-carboxylate:



To a stirred solution of N-methylmorpholine-N-oxide (735 mg, 6.3 mmol) and osmium tetroxide (5 mg, 0.02 mmol) in acetone (30 ml) was added a solution of methyl 5-(3-butenyl)-4-methylthiophene-2-carboxylate (701 mg, 3.3 mmol) in acetone (20 ml). The resultant solution was stirred under an argon atmosphere at ambient temperature for 48 hours, then filtered through a pad of Celite. The filtrate was acidified by addition of 0.5 M H_2SO_4 (10 ml), and the acetone was removed by concentration *in vacuo*. The aqueous residue was diluted with water (20 ml) and extracted with EtOAc (3 x 25 ml). The combined organic extracts were washed with water (3 x 25 ml), dried over Na_2SO_4, and concentrated *in vacuo* to give a brown gum, which was purified by flash chromatography. Elution with CH_2Cl_2:EtOAc (2:3) provided the product as an off-white solid (577 mg, 71% yield). Analyses indicated that the product was methyl 5-(3,4-dihydroxybutyl)-4-methylthiophene-2-carboxylate. NMR ($CDCl_3$) δ: 7.50 (1H, s), 3.84 (3H, s), 3.79 - 3.72 (1H, m), 3.86 (1H, dd, J = 3.2, 10.9 Hz), 3.48 (1H, dd, J = 7.4, 10.9 Hz), 3.00 - 2.80 (2H, m). Anal. ($C_{11}H_{16}O_4S$) C,H,S.

The above examples are given to illustrate various aspects of the invention. It is to be understood that appropriate modifications will be within the capabilities of one having ordinary skill in the art in light of the teachings contained herein.

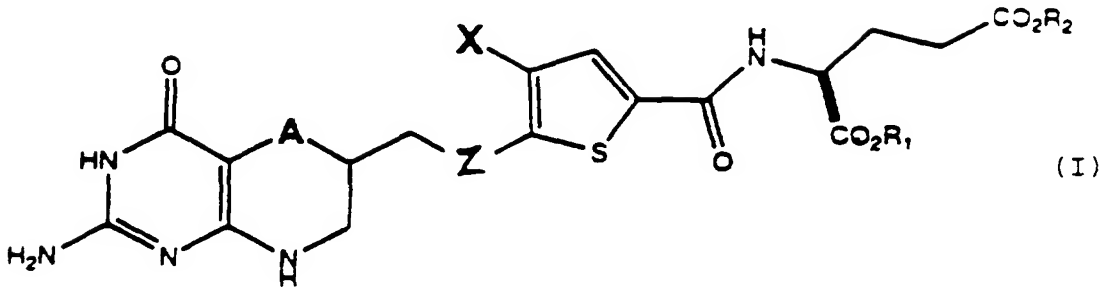
Where possible as a matter of chemistry, chemical groups recited herein can be substituted. In some cases,

this possibility is made explicit by reciting, e.g., substituted or unsubstituted C_1-C_3 alkyl group.

Where more than one R_6 group is recited in any Formula herein, each R_6 can be independently selected from the possibilities given.

WHAT IS CLAIMED IS:

1. A compound of the Formula I:



(I)

wherein:

A is sulfur, CH_2 or selenium;

Z is a substituted or unsubstituted C_1-C_3 alkyl group, a substituted or unsubstituted C_2-C_3 alkenyl group, a substituted or unsubstituted C_2-C_3 alkynyl group, a substituted or unsubstituted amino group, sulfur or oxygen;

X is a substituted or unsubstituted C_1-C_6 alkyl group; a substituted or unsubstituted C_2-C_6 alkenyl group; a substituted or unsubstituted C_2-C_6 alkynyl group; -C(O)E, wherein E is hydrogen, a substituted or unsubstituted C_1-C_3 alkyl group, a substituted or unsubstituted C_2-C_3 alkenyl group, a substituted or unsubstituted C_2-C_3 alkynyl group, a substituted or unsubstituted OC_1-C_3 alkoxy group, or $NR_{10}R_{11}$, wherein R_{10} and R_{11} are independently selected from hydrogen, substituted and unsubstituted C_1-C_3 alkyl groups, substituted and unsubstituted C_2-C_3 alkenyl groups, substituted and unsubstituted C_2-C_3 alkynyl groups; $NR_{10}R_{11}$, wherein R_{10} and R_{11} are independently defined as set forth above; hydroxyl; nitro; SR_{12}, wherein R_{12} is hydrogen, a substituted or unsubstituted C_1-C_6 alkyl group, a substituted or unsubstituted C_2-C_6 alkenyl group, or a substituted or unsubstituted C_2-C_6 alkynyl group; cyano; or a substituted or unsubstituted $O(C_1$-$C_3)$ group; and

R_1 and R_2 are each independently hydrogen or a moiety that forms, together with the attached CO_2, a readily hydrolyzable ester group;
or a pharmaceutically acceptable salt thereof.

2. A compound or salt according to claim 1, wherein A is sulfur or CH_2.

3. A compound or salt according to claim 1, wherein Z is CH_2, CH_2CH_2, NH, oxygen, sulfur, $CH(CH_2OH)$ or NCH_3.

4. A compound or salt according to claim 1, wherein: when X is substituted, the substituents are selected from OH, NH_2, O-methyl, O-ethyl, SH, SCH_3 and NH-methyl; and when Z is substituted, the substituents are selected from C_1-C_6 alkoxyl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2 C_6 alkynyl, acyl, halogen, amino, hydroxyl, nitro, mercapto, monocyclic carbocycle, monocyclic heterocycle, nonfused polycyclic carbocycle, nonfused polycyclic heterocycle, hydroxy C_1-C_6 alkyl, and C_1-C_6 alkoxy C_1-C_6 alkyl.

5. A compound or salt according to claim 1, wherein X is unsubstituted.

6. A compound or salt according to claim 5, wherein X is methyl or ethyl.

7. A compound or salt according to claim 1, wherein R_1 and R_2 each is independently hydrogen, C_1-C_6 alkyl, hydroxyalkyl, alkylaryl or aralkyl.

8. A compound or salt according to claim 7, wherein R_1 and R_2 each is independently hydrogen or C_1-C_2 alkyl.

9. A compound or salt according to claim 8, wherein R_1 and R_2 are each hydrogen.

10. A compound or salt according to claim 1, wherein A is sulfur or CH_2, Z is CH_2, and X is methyl.

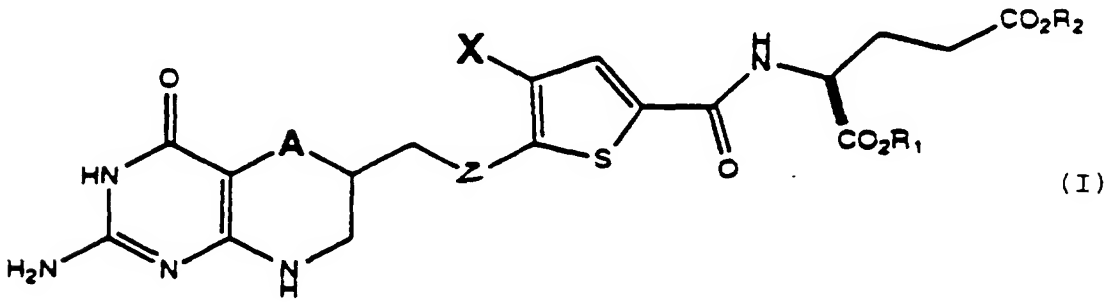
11. A compound or salt according to claim 1, selected from:

N-(5-[2-(2-amino-4(3H)-oxo-5,6,7,8-tetrahydropyrido[2,3-d]-pyrimidin-6-yl)ethyl]-4-methylthieno-2-yl)-L-glutamic acid and its pharmaceutically acceptable salts;
N-(5-[2-(2-amino-4-oxo-4,6,7,8-tetrahydro-3H-pyrimido[5,4-6][1,4]-thiazin-6-yl)ethyl]-4-methylthieno-2-yl)-L-glutamic acid diethyl ester and its pharmaceutically acceptable salts; and N-(5-[2-(2-amino-4-oxo-4,6,7,8-tetrahydro-3H-pyrimido-[5,4-6][1,4]thiazin-6-yl)ethyl]-4-methylthieno-2-yl)-L-glutamic acid and its pharmaceutically acceptable salts.

12. N-(5-[2-(2-amino-4(3H)-oxo-5,6,7,8-tetrahydro-pyrido[2,3-d]pyrimidin-6-yl)ethyl]-4-methylthieno-2-yl)-L-glutamic acid.

13. A pharmaceutical composition comprising:

(i) a compound of the Formula I:



(I)

wherein:

A is sulfur, CH_2 or selenium;

Z is a substituted or unsubstituted C_1-C_3 alkyl group, a substituted or unsubstituted C_2-C_3 alkenyl group, a substituted or unsubstituted C_2-C_3 alkynyl group, a substituted or unsubstituted amino group, sulfur or oxygen;

X is a substituted or unsubstituted C_1-C_6 alkyl group; a substituted or unsubstituted C_2-C_6 alkenyl group; a substituted or unsubstituted C_2-C_6 alkynyl group; -C(O)E, wherein E is hydrogen, a substituted or unsubstituted C_1-C_3 alkyl group, a substituted or unsubstituted C_2-C_3 alkenyl group, a substituted or unsubstituted C_2-C_3 alkynyl group, a substituted or unsubstituted OC_1-C_3 alkoxy group, or

$NR_{10}R_{11}$, wherein R_{10} and R_{11} are independently selected from hydrogen, substituted and unsubstituted C_1-C_3 alkyl groups, substituted and unsubstituted C_2-C_3 alkenyl groups, substituted and unsubstituted C_2-C_3 alkynyl groups; $NR_{10}R_{11}$, wherein R_{10} and R_{11} are independently defined as set forth above; hydroxyl; nitro; SR_{12}, wherein R_{12} is hydrogen, a substituted or unsubstituted C_1-C_6 alkyl group, a substituted or unsubstituted C_2-C_6 alkenyl group, or a substituted or unsubstituted C_2-C_6 alkynyl group; cyano; or a substituted or unsubstituted $O(C_1$-$C_3)$ group; and

R_1 and R_2 are each independently hydrogen or a moiety that forms, together with the attached CO_2, a readily hydrolyzable ester group;

or a pharmaceutically acceptable salt thereof; and

(ii) a pharmaceutically acceptable carrier.

14. A pharmaceutical composition according to claim 13, wherein A is sulfur or CH_2.

15. A pharmaceutical composition according to claim 13, wherein Z is CH_2, CH_2CH_2, NH, oxygen, sulfur, $CH(CH_2OH)$ or NCH_3.

16. A pharmaceutical composition according to claim 13, wherein: when X is substituted, the substituents are selected from OH, NH_2, O-methyl, O-ethyl, SH, SCH_3 and NH-methyl; and when Z is substituted, the substituents are selected from C_1-C_6 alkoxyl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2 C_6 alkynyl, acyl, halogen, amino, hydroxyl, nitro, mercapto, monocyclic carbocycle, monocyclic heterocycle, nonfused polycyclic carbocycle, nonfused polycyclic heterocycle, hydroxy C_1-C_6 alkyl, and C_1-C_6 alkoxy C_1-C_6 alkyl.

17. A pharmaceutical composition according to claim 13, wherein X is unsubstituted.

18. A pharmaceutical composition according to claim 17, wherein X is methyl or ethyl.

19. A pharmaceutical composition according to claim 13, wherein R_1 and R_2 each is independently hydrogen, C_1-C_6 alkyl, hydroxyalkyl, alkylaryl or aralkyl.

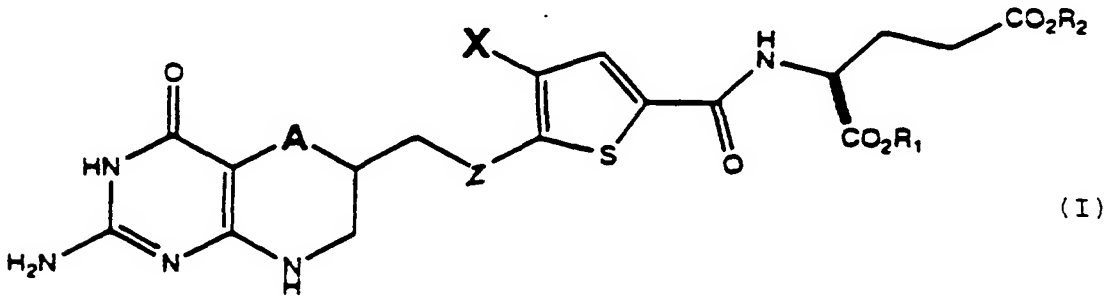
20. A pharmaceutical composition according to claim 19, wherein R_1 and R_2 each is independently hydrogen or C_1-C_2 alkyl.

21. A pharmaceutical composition according to claim 20, wherein R_1 and R_2 are each hydrogen.

22. A pharmaceutical composition according to claim 13, wherein A is sulfur or CH_2, Z is CH_2, and X is methyl.

23. A pharmaceutical composition according to claim 13, wherein said compound of the Formula I is N-(5-[2-(2-amino-4(3H)-oxo-5,6,7,8-tetrahydro-pyrido[2,3-d]pyrimidin-6-yl)ethyl]-4-methylthieno-2-yl)-L-glutamic acid.

24. A method of inhibiting the growth or proliferation of cells of microorganisms or higher organisms, comprising administering to a mammalian or avian host an effective quantity of a compound of the Formula I:



(I)

wherein:

A is sulfur, CH_2 or selenium;

Z is a substituted or unsubstituted C_1-C_3 alkyl group, a substituted or unsubstituted C_2-C_3 alkenyl group, a substituted or unsubstituted C_2-C_3 alkynyl group, a substituted or unsubstituted amino group, sulfur or oxygen;

X is a substituted or unsubstituted C_1-C_6 alkyl group; a substituted or unsubstituted C_2-C_6 alkenyl group; a substituted or unsubstituted C_2-C_6 alkynyl group; -C(O)E, wherein E is hydrogen, a substituted or unsubstituted C_1-C_3 alkyl group, a substituted or unsubstituted C_2-C_3 alkenyl group, a substituted or unsubstituted C_2-C_3 alkynyl group, a substituted or unsubstituted OC_1-C_3 alkoxy group, or $NR_{10}R_{11}$, wherein R_{10} and R_{11} are independently selected from hydrogen, substituted and unsubstituted C_1-C_3 alkyl groups, substituted and unsubstituted C_2-C_3 alkenyl groups, substituted and unsubstituted C_2-C_3 alkynyl groups; $NR_{10}R_{11}$, wherein R_{10} and R_{11} are independently defined as set forth above; hydroxyl; nitro; SR_{12}, wherein R_{12} is hydrogen, a substituted or unsubstituted C_1-C_6 alkyl group, a substituted or unsubstituted C_2-C_6 alkenyl group, or a substituted or unsubstituted C_2-C_6 alkynyl group; cyano; or a substituted or unsubstituted $O(C_1$-$C_3)$ group; and

R_1 and R_2 are each independently hydrogen or a moiety that forms, together with the attached CO_2, a readily hydrolyzable ester group;

or a pharmaceutically acceptable salt thereof.

25. A method according to claim 25, wherein A is sulfur or CH_2.

26. A method according to claim 25, wherein Z is CH_2, CH_2CH_2, NH, oxygen, sulfur, $CH(CH_2OH)$ or NCH_3.

27. A method according to claim 25, wherein: when X is substituted, the substituents are selected from OH, NH_2, O-methyl, O-ethyl, SH, SCH_3 and NH-methyl; and when Z is substituted, the substituents are selected from C_1-C_6 alkoxyl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, acyl, halogen, amino, hydroxyl, nitro, mercapto, monocyclic carbocycle, monocyclic heterocycle, nonfused polycyclic carbocycle, nonfused polycyclic heterocycle, hydroxy C_1-C_6 alkyl, and C_1-C_6 alkoxy C_1-C_6 alkyl.

28. A method according to claim 25, wherein X is unsubstituted.

29. A method according to claim 28, wherein X is methyl or ethyl.

30. A method according to claim 25, wherein R_1 and R_2 each is independently hydrogen, C_1-C_6 alkyl, hydroxyalkyl, alkylaryl or aralkyl.

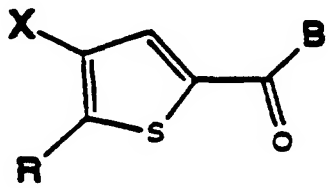
31. A method according to claim 30, wherein R_1 and R_2 each is independently hydrogen or C_1-C_2 alkyl.

32. A method according to claim 31, wherein R_1 and R_2 are each hydrogen.

33. A method according to claim 25, wherein A is sulfur or CH_2, and X is methyl.

34. A method according to claim 25, wherein said compound of the Formula I is N-(5-[2-(2-amino-4(3H)-oxo-5,6,7,8-tetrahydro-pyrido[2,3-d]pyrimidin-6-yl)ethyl]-4-methylthieno-2-yl)-L-glutamic acid.

35. A compound of the Formula II:



(II)

wherein:

R is a halogen;

X is a substituted or unsubstituted C_1-C_6 alkyl group; and

B is an amino acid linked through the amino portion to form an amide, or a C_1-C_6 alcohol linked through the alcohol portion to form an ester.

36. A compound according to claim 35, wherein R is bromo.

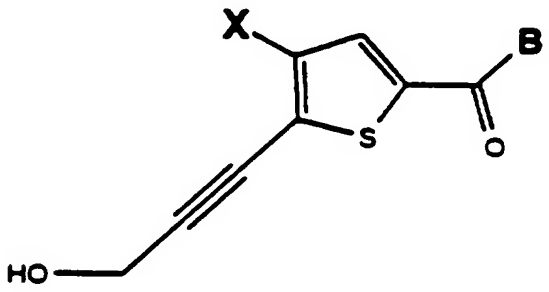
37. A compound according to claim 35, wherein when X is substituted, the substituents are selected from OH, NH_2, O-methyl, O-ethyl, SH, SCH_3 and NH-methyl.

38. A compound according to claim 35, wherein X is unsubstituted.

39. A compound according to claim 38, wherein X is methyl or ethyl.

40. A compound according to claim 35, wherein B is diethyl glutamate or methyl or ethyl alcohol.

41. A compound of the Formula VII:



(VII)

wherein:

X is a substituted or unsubstituted C_1-C_6 alkyl group; and

B is an amino acid linked through the amino portion to form an amide, or a C_1-C_6 alcohol linked through the alcohol portion to form an ester.

42. A compound according to claim 41, wherein when X is substituted, the substituents are selected from OH, NH_2, O-methyl, O-ethyl, SH, SCH_3 and NH-methyl.

43. A compound according to claim 41, wherein X is unsubstituted.

44. A compound according to claim 43, wherein X is methyl or ethyl.

45. A compound according to claim 41, wherein B is diethyl glutamate or methyl or ethyl alcohol.

INTERNATIONAL SEARCH REPORT

Inter[redacted]ation No
PCT/US 95/09519

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D471/04 C07D513/04 A61K31/505 C07D333/38
//(C07D471/04,239:00,221:00),(C07D513/04,279:00,239:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 343 801 (PRINCETON UNIVERSITY) 29 November 1989 see claims 1,16 ---	1,13
X	WO,A,94 13295 (AGOURON) 23 June 1994 see page 40, paragraph 1 - page 41, paragraph 1 ---	35-41, 43-45
X	EP,A,0 109 381 (LAEVOSAN) 23 May 1984 see page 9, line 19 - line 20 ---	35,38-40
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 October 1995	20. 10. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Alfaro Faus, I

Form PCT ISA 210 (second sheet) (July 1992)

C.(Conunuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF THE CHEMICAL SOCIETY, PERKIN TRANSACTIONS 1, 1980 LETCHWORTH GB, pages 1029-1037, K. CLARKE ET AL. 'Condensed isothiazoles. Part 5. Thieno[2,3-d]isothiazoles and thieno[3,2-d]isothiazoles' see compounds IIIa and IV see page 1034, paragraph 3 - paragraph 4 ---	35,38,39
X	COLLECTION OF CZECHOSLOVAK CHEMICAL COMMUNICATIONS, vol. 39, 1974 PRAGUE CS, pages 3527-3531, M. NEMEC ET AL. 'The synthesis of 4-substituted 2-thiophenecarboxylic acids' see compounds IIIa and IV -----	35,36, 38,39

INTERNATIONAL SEARCH REPORT

ernationa[redacted]cation No.
PCT/US 95/09519

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 24- 34 are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Intern. ial Application No
PCT/US 95/09519

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-343801	29-11-89	US-A-	4882333	21-11-89
		US-A-	4882334	21-11-89
		AT-T-	109480	15-08-94
		AU-B-	611027	30-05-91
		AU-B-	3510989	30-11-89
		CN-B-	1025334	06-07-94
		DE-D-	68917211	08-09-94
		DE-T-	68917211	24-11-94
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		JP-A-	59108780	23-06-84
		US-A-	4590203	20-05-86

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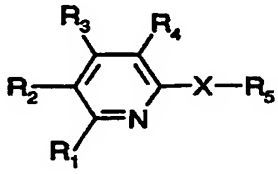
(54) Title: PYRIDINE DERIVATIVES



(I)

(57) Abstract

Compounds of the formula (I), wherein X and R_1 to R_5 are as defined in the description, are useful for treating disorders mediated full or in part by mGluR5.

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Pyridine derivatives

The invention relates to the use of 2-arylalkenyl-, 2-heteroarylalkenyl-, 2-arylalkynyl-, 2-heteroarylalkynyl-, 2-arylazo- and 2-heteroarylazo-pyridines for modulating the activity of mGluRs and for treating mGluR5 mediated diseases, to pharmaceutical compositions for use in such therapy, as well as to novel 2-arylalkenyl-, 2-heteroarylalkenyl-, 2-arylalkynyl-, 2-heteroarylalkynyl-, 2-arylazo- and 2-heteroarylazo-pyridines.

It has been found that 2-arylalkenyl-, 2-heteroarylalkenyl-, 2-arylalkynyl-, 2-heteroarylalkynyl-, 2-arylazo- and 2-heteroarylazo-pyridines including the pharmaceutically acceptable salts (hereinafter agents of the invention) are useful as modulators of mGluRs. Modulation of mGluRs can be demonstrated in a variety of ways, inter alia, in binding assays and functional assays such as second messenger assays or measurement of changes in intracellular calcium concentrations. For example, measurement of the inositol phosphate turnover in recombinant cell lines expressing hmGluR5a showed, for selected agents of the invention, IC_{50} values of about 1nM to about 50μM.

In particular, the agents of the invention have valuable pharmacological properties. For example, they exhibit a marked and selective modulating, especially antagonistic, action at human metabotropic glutamate receptors (mGluRs). This can be determined in vitro for example at recombinant human metabotropic glutamate receptors, especially PLC-coupled subtypes thereof such as mGluR5, using different procedures like, for example, measurement of the inhibition of the agonist induced elevation of intracellular Ca^{2+} concentration in accordance with L. P. Daggett et al. Neuropharm. Vol. 34, pages 871-886 (1995), P. J. Flor et al., J. Neurochem. Vol. 67, pages 58-63 (1996) or by determination to what extent the agonist induced elevation of the inositol phosphate turnover is inhibited as described by T. Knoepfel et al. Eur. J. Pharmacol. Vol. 288, pages 389-392 (1994), L. P. Daggett et al., Neuropharm. Vol. 67, pages 58-63 (1996) references cited therein. Isolation and expression of human mGluR subtypes are described in US-Patent No. 5,521,297. Selected agents of the invention showed IC_{50} values for the inhibition of the quisqualate-induced inositol phosphate turnover, measured in recombinant cells expressing hmGluR5a of about 1nM to about 50μM.

Accordingly the invention relates to agents of the invention for use in the treatment of disorders associated with irregularities of the glutamatergic signal transmission, and of nervous system disorders mediated full or in part by mGluR5.

Disorders associated with irregularities of the glutamatergic signal transmission are for example epilepsy, cerebral ischemias, especially acute ischemias, ischemic diseases of the eye, muscle spasms such as local or general spasticity and, in particular, convulsions or pain.

Nervous system disorders mediated full or in part by mGluR5 are for example acute, traumatic and chronic degenerative processes of the nervous system, such as Parkinson's disease, senile dementia, Alzheimer's disease, Huntington's chorea, amyotrophic lateral sclerosis and multiple sclerosis, psychiatric diseases such as schizophrenia and anxiety, depression and pain.

The invention also relates to the use of agents of the invention, in the treatment of disorders associated with irregularities of the glutamatergic signal transmission, and of nervous system disorders mediated full or in part by Group I mGluRs.

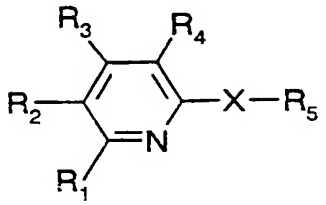
Furthermore the invention relates to the use of agents of the invention for the manufacture of a pharmaceutical composition designed for the treatment of disorders associated with irregularities of the glutamatergic signal transmission, and of nervous system disorders mediated full or in part by Group I mGluRs.

In a further aspect the invention relates to a method of treating disorders mediated full or in part by group I mGluRs (preferentially mGluR5) which method comprises administering to a warm-blooded organism in need of such treatment a therapeutically effective amount of an agent of the invention.

In still a further aspect, the invention relates to novel 2-arylalkenyl-, 2-heteroarylalkenyl-, 2-arylalkynyl-, 2-heteroarylalkynyl-, 2-arylazo- and 2-heteroarylazo-pyridines and their salts, and to a process for preparing them.

Moreover the invention relates to a pharmaceutical composition comprising as pharmaceutical active ingredient, together with customary pharmaceutical excipients, a novel 2-arylalkenyl-, 2-heteroarylalkenyl-, 2-arylalkynyl-, 2-heteroarylalkynyl-, 2-arylazo- or 2-heteroarylazo-pyridine or a pharmaceutically acceptable salt thereof.

Agents of the invention are for example compounds of formula I



(I),

wherein

R_1 denotes hydrogen, lower alkyl, hydroxy-lower alkyl lower alkyl-amino, piperidino, carboxy, esterified carboxy, amidated carboxy, unsubstituted or lower alkyl-, lower alkoxy-, halo- and/or trifluoromethyl-substituted N-lower-alkyl-N-phenylcarbamoyl, lower alkoxy, halo-lower alkyl or halo-lower alkoxy,

R_2 denotes hydrogen, lower alkyl, carboxy, esterified carboxy, amidated carboxy, hydroxy-lower alkyl, hydroxy, lower alkoxy or lower alkanoyloxy, 4-(4-fluoro-benzoyl)-piperidin-1-yl-carboxy, 4-t.-butyloxycarbonyl-piperazin-1-yl-carboxy, 4-(4-azido-2-hydroxybenzoyl)-piperazin-1-yl-carboxy or 4-(4-azido-2-hydroxy-3-iodo-benzoyl)-piperazin-1-yl-carboxy,

R_3 represents hydrogen, lower alkyl, carboxy, lower alkoxy-carbonyl, lower alkyl-carbamoyl, hydroxy- lower alkyl, di- lower alkyl- aminomethyl, morpholinocarbonyl or 4-(4-fluoro-benzoyl)-piperidin-1-yl-carboxy,

R_4 represents hydrogen, lower alkyl, hydroxy, hydroxy-lower alkyl, amino-lower alkyl, lower alkylamino-lower alkyl, di-lower alkylamino-lower alkyl, unsubstituted or hydroxy-substituted lower alkyleneamino-lower alkyl, lower alkoxy, lower alkanoyloxy, amino-lower alkoxy, lower alkylamino-lower alkoxy, di-lower alkylamino-lower alkoxy, phthalimido-lower alkoxy, unsubstituted or hydroxy- or 2-oxo-imidazolidin-1-yl-substituted lower alkyleneamino-lower alkoxy, carboxy, esterified or amidated carboxy, carboxy-lower-alkoxy or esterified carboxy-lower-alkoxy,

X represents an optionally halo-substituted lower alkenylene or alkynylene group bonded via vicinal unsaturated carbon atoms or an azo (-N=N-) group, and

R_5 denotes an aromatic or heteroaromatic group which is unsubstituted or substituted by one or more substituents selected from lower alkyl, halo, halo-lower alkyl, halo-lower alkoxy, lower alkenyl, lower alkynyl, unsubstituted or lower alkyl-, lower alkoxy-, halo- and/or trifluoromethyl-substituted phenyl, unsubstituted or lower alkyl-, lower alkoxy-, halo- and/or trifluoromethyl-substituted phenyl-lower alkynyl, hydroxy, hydroxy-lower alkyl, lower alkanoyloxy-lower alkyl, lower alkoxy, lower alkenyloxy, lower alkylenedioxy, lower alkanoyloxy, amino-, lower alkylamino-, lower alkanoylamino- or N-lower alkyl-N-lower alkanoylamino-lower alkoxy, unsubstituted or lower alkyl- lower alkoxy-, halo- and/or trifluoromethyl-substituted phenoxy, unsubstituted or lower alkyl- lower alkoxy-, halo- and/or

trifluoromethyl-substituted phenyl-lower alkoxy, acyl, carboxy, esterified carboxy, amidated carboxy, cyano, carboxy-lower alkylamino, esterified carboxy-lower alkylamino, amidated carboxy-lower alkylamino, phosphono-lower alkylamino, esterified phosphono-lower alkylamino, nitro, amino, lower alkylamino, di-lower alkylamino, acylamino, N-acyl-N-lower alkylamino, phenylamino, phenyl-lower alkylamino, cycloalkyl-lower alkylamino or heteroaryl-lower alkylamino each of which may be unsubstituted or lower alkyl- lower alkoxy-, halo- and/or trifluoromethyl-substituted,
customary photoaffinity ligands and customary radioactive markers, inclusive of their N-oxides and their pharmaceutically acceptable salts.

Compounds of formula I having basic groups may form acid addition salts, and compounds of the formula I having acidic groups may form salts with bases. Compounds of formula I having basic groups and in addition having at least one acidic group, may also form internal salts.

Also included are both total and partial salts, that is to say salts with 1, 2 or 3, preferably 2, equivalents of base per mole of acid of formula I, or salts with 1, 2 or 3 equivalents, preferably 1 equivalent, of acid per mole of base of formula I.

For the purposes of isolation or purification it is also possible to use pharmaceutically unacceptable salts. Only the pharmaceutically acceptable, non-toxic salts are used therapeutically and they are therefore preferred.

Halo in the present description denotes fluorine, chlorine, bromine or iodine.

When X represents an alkenylene group, configuration trans is preferred.

Preferred compounds of formula I are those wherein

X represents an optionally halo-substituted (C_{2-4})alkenylene or alkynylene group bonded via vicinal unsaturated carbon atoms,

R_1 is hydrogen, (C_{1-4}) alkyl, (C_{1-4})alkoxy, hydroxy(C_{1-4})alkyl, cyano, ethynyl, carboxy, (C_{1-4})alkoxycarbonyl, di(C_{1-4})alkylamino, (C_{1-6})alkylaminocarbonyl, trifluoromethylphenylaminocarbonyl,

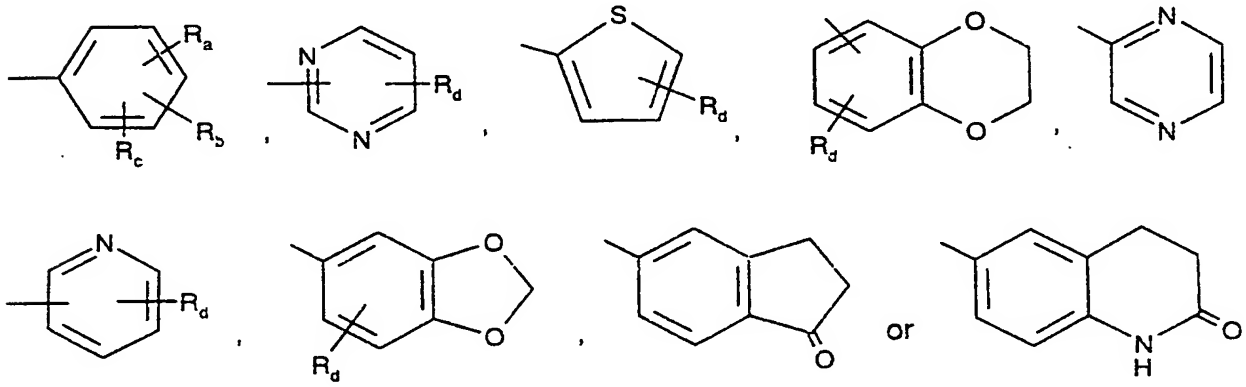
R_2 is hydrogen, hydroxy, (C_{1-4}) alkyl, hydroxy (C_{1-4}) alkyl, (C_{1-4}) alkoxy, carboxy, (C_{2-5})alkanoyloxy, (C_{1-4}) alkoxycarbonyl, di(C_{1-4})alkylamino(C_{1-4})alkanoyl,

di(C_{1-4})alkylaminomethyl, 4-(4-fluoro-benzoyl)-piperidin-1-yl-carboxy, 4-t.-butyloxycarbonyl-piperazin-1-yl-carboxy, 4-(4-azido-2-hydroxybenzoyl)-piperazin-1-yl-carboxy or 4-(4-azido-2-hydroxy-3-iodo-benzoyl)-piperazin-1-yl-carboxy,

R_3 is hydrogen, (C_{1-4}) alkyl, carboxy, (C_{1-4})alkoxycarbonyl, (C_{1-4})alkylcarbamoyl, hydroxy(C_{1-4})alkyl, di(C_{1-4})alkylaminomethyl, morpholinocarbonyl or 4-(4-fluoro-benzoyl)-piperidin-1-yl-carboxy,

R_4 is hydrogen, hydroxy, (C_{1-4})alkoxy, carboxy, (C_{2-5})alkanoyloxy, (C_{1-4})alkoxycarbonyl, amino(C_{1-4})alkoxy, di(C_{1-4})alkylamino(C_{1-4})alkoxy, di(C_{1-4})alkylamino(C_{1-4})alkyl, carboxy (C_{1-4})alkylcarbonyl, (C_{1-4})alkoxycarbonyl(C_{1-4})alkoxy, hydroxy(C_{1-4})alkyl, di(C_{1-4})alkylamino(C_{1-4})alkoxy, m-hydroxy-p-azidophenylcarbonylamino(C_{1-4})alkoxy, and

R_5 is a group of formula



wherein

R_a and R_b independently are hydrogen, hydroxy, halogen, nitro, cyano, carboxy, (C_{1-4})alkyl, (C_{1-4})alkoxy, hydroxy(C_{1-4})alkyl, (C_{1-4})alkoxycarbonyl, (C_{2-7})alkanoyl, (C_{2-5})alkanoyloxy, (C_{2-5})alkanoyloxy(C_{1-4})alkyl, trifluoromethyl, trifluoromethoxy, trimethylsilylethynyl, (C_{2-5})alkynyl, amino, azido, amino (C_{1-4})alkoxy, (C_{2-5})alkanoylamino(C_{1-4})alkoxy, (C_{1-4})alkylamino(C_{1-4})alkoxy, di(C_{1-4})alkylamino (C_{1-4})alkoxy, (C_{1-4})alkylamino, di(C_{1-4})alkylamino, monohalobenzylamino, thienylmethylamino, thienylcarbonylamino, trifluoromethylphenylaminocarbonyl, tetrazolyl, (C_{2-5})alkanoylamino, benzylcarbonylamino, (C_{1-4})alkylaminocarbonylamino, (C_{1-4})alkoxycarbonyl-aminocarbonylamino or (C_{1-4})alkylsulfonyl,

R_c is hydrogen, fluorine, chlorine, bromine, hydroxy, (C_{1-4})alkyl, (C_{2-5})alkanoyloxy, (C_{1-4})alkoxy or cyano, and

R_d is hydrogen, halogen or (C_{1-4})alkyl.

More preferred compounds of formula I are those wherein X is as defined above and

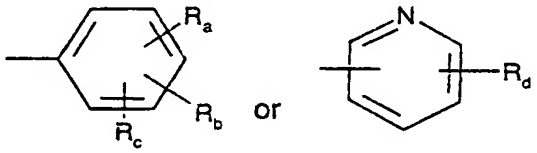
R_1 is hydrogen, (C_{1-4}) alkyl, (C_{1-4})alkoxy, cyano, ethynyl or di(C_{1-4})alkylamino,

R_2 is hydrogen, hydroxy, carboxy, (C_{1-4}) alkoxycarbonyl, di(C_{1-4})alkylaminomethyl, 4-(4-fluoro-benzoyl)-piperidin-1-yl-carboxy, 4-t.-butyloxycarbonyl-piperazin-1-yl-carboxy, 4-(4-azido-2-hydroxybenzoyl)-piperazin-1-yl-carboxy or 4-(4-azido-2-hydroxy-3-iodo-benzoyl)-piperazin-1-yl-carboxy,

R_3 is as defined above,

R_4 is hydrogen, hydroxy, carboxy, (C_{2-5})alkanoyloxy, (C_{1-4})alkoxycarbonyl, amino (C_{1-4})alkoxy, di(C_{1-4})alkylamino(C_{1-4})alkoxy, di(C_{1-4})alkylamino(C_{1-4})alkyl or hydroxy(C_{1-4})alkyl, and

R_5 is a group of formula



wherein

R_a and R_b independently are hydrogen, halogen, nitro, cyano, (C_{1-4})alkyl, (C_{1-4})alkoxy, trifluoromethyl, trifluoromethoxy or (C_{2-5})alkynyl, and

R_c and R_d are as defined above.

The agents of the invention include, for example, the compounds described in the examples hereinafter.

The usefulness of the agents of the invention in the treatment of the above-mentioned disorders could be confirmed in a range of standard tests including those indicated below:

At doses of about 10 to 100 mg/kg i.p. or p.o. with pretreatment times of 15 min. to 8 hours, the agents of the invention show anticonvulsive activity in the electroshock induced convulsion model [cf. E.A. Swinyard, J. Pharm. Assoc. Scient. Ed. 38, 201 (1949) and J. Pharmacol. Exptl. Therap. 106, 319 (1952)].

At doses of about 4 to about 40 mg/kg p.o., the agents of the invention show reversal of Freund complete adjuvant (FCA) induced hyperalgesia [cf. J. Donnerer et al., Neuroscience 49, 693-698 (1992) and C.J. Woolf, Neuroscience 62, 327-331 (1994)].

For all the above mentioned indications, the appropriate dosage will of course vary depending upon, for example, the compound employed, the host, the mode of administration and the nature and severity of the condition being treated. However, in general, satisfactory results in animals are indicated to be obtained at a daily dosage of from about 0.5 to about 100 mg/kg animal body weight. In larger mammals, for example humans, an indicated daily dosage is in the range from about 5 to 1500 mg, preferably about 10 to about 1000 mg of the compound conveniently administered in divided doses up to 4 times a day or in sustained release form.

Preferred compounds for the above mentioned indications include (3-{2-[2-trans-(3,5-dichlorophenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethylamine (A), 2-methyl-6-styryl-pyridine (B), 2-(3-fluoro-phenylethynyl)-6-methyl-pyridine (C) and 2-(4-ethoxy-3-trifluoromethyl-phenylethynyl)-6-methyl-pyridine (D). It has for example been determined that in the above-mentioned electroshock induced convulsion model, compounds A and B show anticonvulsive activity with ED_{50}s of 30 and 35 mg/kg i.p. respectively (pretreatment times: 4 hours and 15 min. respectively) and that in the above-mentioned FCA induced hyperalgesia model, compounds C and D show reversal of the hyperalgesia with ED_{50}s of 4.2 and 19 mg/kg p.o. respectively (post-treatment time: 3 hours).

As indicated above, the agents of the invention include novel 2-arylalkenyl-, 2-heteroarylalkenyl-, 2-arylalkynyl-, 2-heteroarylalkynyl-, 2-arylazo- and 2-heteroarylazo-pyridines and their salts, hereinafter referred to as "compounds of the invention".

Compounds of the invention include compounds of formula I as defined above, and their salts, wherein X and R_1 to R_5 are as defined above, provided that when R_3 is hydrogen, a) in compounds of the formula I in which R_1, R_2 and R_4 are hydrogen, R_5 is different from phenyl, monohalophenyl, 2,4- and 3,4-dichlorophenyl, 3- and 4-trifluoromethylphenyl, methylphenyl, 3,4- and 2,5-dimethylphenyl, 4-isopropylphenyl, 3,5-di-tert.-butylphenyl, methoxyphenyl, 3,4-dimethoxyphenyl, 2,4,5- and 3,4,5-trimethoxyphenyl, hydroxyphenyl, 3,5-dihydroxyphenyl, 4-hydroxy-3,5-dimethyl-phenyl, 3-hydroxy-4-methoxy- and 4-hydroxy-3-methoxy-phenyl, 4-hydroxy-(3-methyl-5-tert.-butyl-, 2- and 4-acetylaminophenyl, 3,5-diisopropyl- and 3,5-di-tert.-butyl)phenyl, 4-carboxy- and 4-ethoxycarbonylphenyl, 4-cyanophenyl, 3-methoxycarbonylphenyl, 3-carboxy-5-methoxy-phenyl, 2-pyridinyl, 5-chloro-2-pyridinyl and 6-methyl-2-pyridinyl when X denotes ethenylene, or R_5 is different from phenyl, 4-methylphenyl, 4-methoxyphenyl, 4-bromophenyl and 2- and 4-chlorophenyl when

X denotes 1,2-propylene attached to R_5 in 2-position, or R_5 is different from phenyl, 2- and 4-chlorophenyl and 3-methoxyphenyl when X denotes 1,2-propylene attached to R_5 in 1-position, or R_5 is different from 4-methoxyphenyl when X denotes 2,3-but-2-enylene or 1,2-but-1-enylene attached to R_5 in 2-position, or R_5 is different from 4-methoxyphenyl and 4-isopropyphenyl when X denotes 2,3-pent-2-enylene attached to R_5 in 3-position, or R_5 is different from phenyl, 4-methylphenyl, methoxyphenyl and 4-hydroxyphenyl when X denotes 3,4-hex-3-enylene;

b) in compounds of the formula I in which R_1 is methyl and R_2 and R_4 are hydrogen, R_5 is different from phenyl, 3-methylphenyl, 2-methoxyphenyl, 2-chlorophenyl, 4-cyanophenyl, , 2-pyridinyl and 6-methyl-2-pyridinyl when X denotes ethenylene;

c) in compounds of the formula I in which R_1 and R_2 are hydrogen and R_4 is carboxy, R_5 is different from phenyl, 3-methylphenyl, 4-methoxyphenyl and 4-bromophenyl when X denotes ethenylene;

d) in compounds of the formula I in which R_1 and R_2 are hydrogen and R_4 is methyl, R_5 is different from phenyl, 3-methoxy-, 4-methoxy- and 3,4-dimethoxyphenyl, 2-chloro- and 2,4-dichlorophenyl and 6-methyl-pyrid-2yl when X denotes ethenylene or R_5 is different from phenyl when X is 1,2-prop-1-enylene attached to R_5 in 2-position;

e) in compounds of the formula I wherein R_1 and R_2 are hydrogen and R_4 is 2-dimethyl-aminoethoxycarbonyl or 3-dimethylaminopropyloxycarbonyl, R_5 is different from 4-methoxy-phenyl when X denotes ethenylene;

f) in compounds of the formula I in which R_1 and R_2 are hydrogen and R_4 is 2-dimethoxy-ethoxy, R_5 is different from phenyl, 4-methylphenyl and 4-methoxycarbonylphenyl when X denotes ethenylene;

g) R_5 is different from phenyl when R_1 and R_2 are hydrogen and R_4 is hydroxy or ethoxy-carbonyl, or when R_1 and R_2 are hydrogen and R_4 is hydroxy, or when R_1 is methyl, R_2 is hydrogen and R_4 is methoxy, or R_1 is but-1-enyl, R_2 is hydrogen and R_4 is hydrogen, or R_1 is hydrogen and R_4 is 2-dimethoxyethoxy, and X is, in each case, ethenylene,

and provided that, when R_3 is hydrogen and X is ethynylene,

a') R_5 is different from phenyl, 2- and 4-nitrophenyl, 4-aminophenyl, 4-chlorophenyl, 4-methylphenyl, 4-methoxyphenyl, 4-ethoxycarbonylphenyl, 5-formyl-2-methoxy-phenyl, 5-carboxy-2-methyo-phenyl and pyridyl when R_1, R_2 and R_4 are hydrogen;

b') in compounds of the formula I in which R_2 and R_4 are hydrogen, R_5 is different from phenyl, 3-methylphenyl. 6-methylpyridin-2-yl and 2-methoxyphenyl when R_1 is methyl, R_5 is different form 6-bromopyridin-2-yl when R_1 is bromo, and R_5 is different form 6-hexyloxypyridin-2-yl when R_1 denotes hexyloxy;

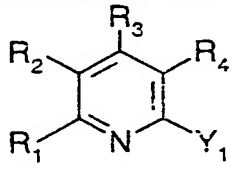
c') in compounds of the formula I wherein R_1 and R_4 are hydrogen, R_5 is different from phenyl, 4-aminophenyl and 4-propylphenyl when R_2 is methyl, R_5 is different from phenyl, 4-cyanophenyl and 4-pentylphenyl when R_2 is ethyl, R_5 is different form 3-cyano-4-ethoxy-phenyland 3-bromo-4-methoxy-phenyl when R_2 is butyl, R_5 is different from 4-methoxy-phenyl and 4 butyloxyphenyl when R_2 is pentyl, R_5 is different form 4-ter.-butylphenyl, 3-tert.-butyl-4-hydroxy-phenyl, 4-tert.-butyl-3-hydroxy-phenyl,and 4-hexyloxyphenyl when R_2 is carboxy, R_5 is different from phenyl when R_2 is methoxycarbonyl or methylcarbamoyl, R_4 is different form 3-tert.-butylphenyl, 3-tert.-butyl-4-hydroxy-phenyl and 4-(4-methylpentyl)phenyl when R_2 is ethoxycarbonyl, and R_5 is different from 4-pentyloxyphenyl when R_2 is 2-methylbutyloxycarbonyl;
d') in compounds of the formula I wherein R_1 and R_2 are hydrogen, R_5 is different from phenyl when R_4 is hydroxy, methyl, ethyl, carboxy, methoxycarbonyl or carbamoyl.

Preferred compounds of the invention are as indicated above for the agents of the invention.

The compounds of the invention can be prepared in analogy to the synthesis of known compounds of formula I.

Thus the compounds of the invention which are of formula I can be prepared for example by a process which comprises

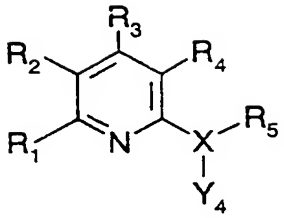
a) reacting a compound of the formula II



(II)

with a compound of the formula $Y_2 — R_5$ (III), in which either one of Y_1 and Y_2 denotes lower alkanoyl and the other one represents lower alkyl or triarylphosphoranylidenemethyl, or one of Y_1 and Y_2 denotes a reactive esterified hydroxy group and the other one represents a group $Y_3 — X$- in which Y_3 is hydrogen or a metallic group, and R_1, R_2, R_3, R_4 and R_5 have the meanings indicated hereinbefore and functional groups R_1, R_2, R_3 and R_4 as well as functional substituents of R_5 may be temporarily protected, or

b) eliminating $H — Y_4$ from a compound of the formula IV



(IV),

in which Y_4 denotes an electrofugal group and R_1, R_2, R_3, R_4, X and R_5 have the meanings indicated hereinbefore and functional groups R_1, R_2, R_3 and R_4 as well as functional substituents of R_5 may temporarily be protected, removing any temporary protecting groups

and, if desired, converting a compound of formula I obtainable by the above-defined processes into a different compound of formula I, resolving a mixture of isomers that may be obtained into the individual isomers and/or converting a compound of formula I having at least one salt-forming group obtainable by the above-defined processes into a salt, or converting a salt obtainable by the above-defined processes into the corresponding free compound or into a different salt.

A lower alkanoyl Y_2 or, more preferably, Y_1 group is, for example, a C_1-C_3alkanoyl group, such as formyl, acetyl or propionyl, especially formyl. A lower alkyl group Y_1 or, more preferably, Y_2 is, for example, a C_1-C_3alkyl group, such as methyl, ethyl or propyl, especially methyl. Triarylphosphoranylidenemethyl Y_2 or, more preferably, Y_1 is, for example, triphenylphosphoranylidenemethyl.

When one of Y_1 and Y_2 denotes a reactive esterified hydroxy group and the other one represents a group of the formula Y_3–X- in which Y_3 denotes hydrogen, the condensation is preferably performed according to the Heck coupling method, for example, in the presence of copper or of a copper catalyst or of a noble metal/phosphine catalyst, such as Palladium or a PdII salt in the presence of triaryl phosphine, for example, Palladium acetate, and of triphenylphosphine, or in the presence of bis-triphenylphosphine-palladium dichloride, preferably in the presence of a tri-lower alkyl amine, for example, trimethylamine, advantageously in the presence of Cu^{I}-I, in a polar organic solvent such as N,N-di-lower alkyl-alkanoic acid amide, for example, dimethylformamide, a di-lower alkyl sulfoxide, for example, dimethylsulfoxide, or dioxan, at temperatures from appropriately 15° C to appropriately 120° C, preferably at the boil.

When one of Y_1 and Y_2 denotes a reactive esterified hydroxy group and the other one represents a group of the formula Y_3–X- in which Y_3 denotes a metallic group such as a

halo-magnesium group, the reaction is preferably performed according to Grignard method, wherein the metallic intermediate is preferably formed *in situ*.

When one of Y_1 and Y_2 denotes lower alkanoyl and the other one represents lower alkyl, the intermolecular condensation of compounds of the formulae II and III is preferably performed according to the Shaw and Wagstaff method or one of its many modifications.

When one of Y_1 and Y_2 denotes lower alkanoyl and the other one represents triarylphosphoranylidenemethyl , the condensation is preferably performed according to the well known Wittig olefin-building method, preferably by forming the phosphoranylidene component from a corresponding triarylphosphonium halide *in situ*, for example, by reacting the latter with a metal base, such as an alkalimetal hydride, such as sodium hydride, or with a metal-organic base, such as a lower alkyl metal compound, such as butyllithium, or with an alkali metal alkanolate, for example, potassium tertiary butoxide, preferably in an inert organic solvent, such as an aromatic or arylaliphatic hydrocarbon, for example, benzene or toluene, at appropriately -10° C to appropriately 39° C, preferably first at 0° to 10° C and then at ambient temperature.

Electrofugal groups Y_4 are, for example, esterified hydroxy groups, such as hydroxy groups esterified with an organic acid, for example, lower alkanoyloxy or hydroxy groups esterified with an anorganic acid, for example, halo groups, or tertiary amino groups, such as tri-lower alkylamino groups, for example, trimethylamino, or lower-alkyleneamino, lower azaalkyleneamino, lower-oxyalkyleneamino or lower thiaalkyleneamino groups, such as pyrrolidino, piperidino, morpholino or thiomorpholino, or corresponding quaternary ammonium groups.

The protection of functional groups by such protecting groups, the protecting groups themselves and the reactions for their removal are described, for example, in standard works.

The elimination of H —Y_4 from compounds of formula IV can be performed in a customary manner. Thus, water or lower alkanoic acids may be eliminated by means of azeotropic distillation, for example, in toluene, advantageously under mild-acidic conditions. Hydrogen halides may be removed under basic conditions such as reaction with an alkalimetal alkanolate, preferably in the corresponding lower alkanol as a solvent or co-solvent, or by heating in the presence of a tertiary amine, such as a tri-lower alkylamine.

The starting materials for the above described reactions are generally known. Novel starting materials can be obtained in manner analogous to the methods for the preparation of known starting materials.

Compounds of formula I obtainable in accordance with the process can be converted into different compounds of formula I in customary manner, for example a free carboxy group may be esterified or amidated, an esterified or amidated carboxy group may be converted into a free carboxy group, an esterified carboxy group can be converted into an unsubstituted or substituted carbamoyl group, a free amino group can be acylated or alkylated, and a free hydroxy can be acylated.

Also, compounds of the formula I can be oxidized by customary methods such as reaction with an organic peroxy acid, yielding the corresponding pyridine-N-oxide derivatives.

Salts of compounds of formula I can also be converted in a manner known *per se* into the free compounds, for example by treatment with a base or with an acid.

Resulting salts can be converted into different salts in a manner known *per se*.

The compounds of formula I, including their salts, may also be obtained in the form of hydrates or may include the solvent used for crystallization.

As a result of the close relationship between the novel compounds in free form and in the form of their salts, hereinbefore and hereinafter any reference to the free compounds and their salts is to be understood as including the free compounds, as well as the corresponding salts.

In a compound of formula I the configuration at individual chirality centers can be selectively reversed. For example, the configuration of asymmetric carbon atoms that carry nucleophilic substituents, such as amino or hydroxy, can be reversed by second order nucleophilic substitution, optionally after conversion of the bonded nucleophilic substituent into a suitable nucleofugal leaving group and reaction with a reagent introducing the original substituent, or the configuration at carbon atoms having hydroxy groups can be reversed by oxidation and reduction, analogously to European Patent Application EP-A-0 236 734.

The invention relates also to pharmaceutical compositions comprising compounds of formula I.

The pharmacologically acceptable compounds of the present invention may be used, for example, in the preparation of pharmaceutical compositions that comprise an effective amount of the active ingredient together or in a mixture with a significant amount of inorganic or organic, solid or liquid, pharmaceutically acceptable carriers.

The pharmaceutical compositions according to the invention are compositions for enteral, such as nasal, rectal or oral, or parenteral, such as intramuscular or intravenous, administration to warm-blooded animals (human beings and animals) that comprise an effective dose of the pharmacological active ingredient alone or together with a significant amount of a pharmaceutically acceptable carrier. The dose of the active ingredient depends on the species of warm-blooded animal, body weight, age and individual condition, individual pharmacokinetic data, the disease to be treated and the mode of administration.

The pharmaceutical compositions comprise from approximately 1% to approximately 95%, preferably from approximately 20% to approximately 90%, active ingredient. Pharmaceutical compositions according to the invention may be, for example, in unit dose form, such as in the form of ampoules, vials, suppositories, dragées, tablets or capsules.

The pharmaceutical compositions of the present invention are prepared in a manner known *per se*, for example by means of conventional dissolving, lyophilizing, mixing, granulating or confectioning processes.

The doses to be administered to warm-blooded animals, for example human beings, of, for example, approximately 70 kg body weight, especially the doses effective in disorders caused by or associated with irregularities of the glutamatergic signal transmission, are from approximately 3 mg to approximately 3 g, preferably from approximately 10 mg to approximately 1 g, for example approximately from 20 mg to 500 mg, per person per day, divided preferably into 1 to 4 single doses which may, for example, be of the same size. Usually, children receive about half of the adult dose. The dose necessary for each individual can be monitored, for example by measuring the serum concentration of the active ingredient, and adjusted to an optimum level.

The following non-limiting Examples serve to illustrate the invention; temperatures are given in degrees Celsius, pressures in mbar.

EXAMPLE 1

3-[2-(6-Methylpyridin-2-yl)-vinyl]-benzonitrile

A solution of 2,6-dimethyl pyridine (4.2ml, 36.28 mMol), 3-cyanobenzaldehyde (4.95g, 37.74 mMol) in acetic anhydride (6.85 ml) is heated under reflux for 16 hours. The acetic anhydride is then evaporated in vacuo and the residue purified on column chromatography (silica gel 400g). The column is first eluted with toluene (400 ml) and then with toluene/ethyl acetate 95:5. The fractions containing the desired compound are combined, evaporated in vacuo. The solid residue is recrystallized from methylene chloride/hexane and 3.18 g of white crystals are isolated. (melting point: 91-92°).

EXAMPLE 2:

2-[2-(6-Methyl-pyridin-2-yl)-vinyl]-benzonitrile

A solution of 2,6-dimethyl pyridine (5.8 ml, 50 mMol), 2-cyanobenzaldehyde (6.81 g, 52 mMol) in acetic anhydride (9.5 ml) is heated under reflux for 16 hours. The acetic anhydride is then evaporated in vacuo and the residue purified on column chromatography (silica gel 400g). The column is first eluted with toluene (400 ml) and then with toluene/ethyl acetate 95:5. The fractions containing the desired compound are combined, evaporated in vacuo. The solid residue is recrystallized from methylene chloride/diisopropyl ether and white crystals are isolated. (melting point: 113-114°).

EXAMPLE 3

2-Methyl-6-[2-(pyridin-4-yl)-vinyl]-pyridine

A solution of 2,6-dimethyl pyridine (5.8 ml, 50 mMol), pyridine-4-carbaldehyde (4.9 ml, 52 mMol) in acetic anhydride (9.5 ml) is heated under reflux for 16 hours. The acetic anhydride is then evaporated in vacuo and the residue purified on column chromatography (silica gel 900g). The column is first eluted with toluene/acetone 4:1 (5 L), then with toluene/acetone 3:1 (5 L) and finally with toluene/acetone 2:1 (15 L). The fractions containing the desired compound are combined, evaporated in vacuo. The solid residue is recrystallized from methylene chloride/diisopropyl ether and 0.956 g of white crystals are isolated. (melting point: 72-73°C).

EXAMPLE 4

2-Methyl-6-[2-(pyridin-3-yl)-vinyl]-pyridine

A solution of 2,6-dimethyl pyridine (5.8 ml, 50 mMol), pyridine-3-carbaldehyde (4.9 ml, 52 mMol) in acetic anhydride (9.5 ml) is heated under reflux for 10 hours. The acetic anhydride is then evaporated in vacuo and the residue purified on column chromatography anhydride is then evaporated in vacuo and the residue purified on column chromatography (silica gel 900g). The column is first eluted with toluene/acetone 9:1 (7 L), then with toluene/acetone 4:1 (5 L) and finally with toluene/acetone 2:1 (5 L). The fractions containing the desired compound are combined, evaporated in vacuo. The solid residue is recrystallized from methylene chloride/diisopropyl ether and 4.28 g of a colorless oil which solidify upon standing at 6-8°C.

EXAMPLE 5

2-[2-(3-Bromophenyl)ethynyl]-6-methyl-pyridine

1.2 g (2.8 mMol) of 2-[1,2-dibromo-2-(3-bromophenyl)-ethyl]-6-methyl-pyridine are dissolved in 10 ml of ethanol. 0.9 g (16.1 mMol) of potassium hydroxide (powder) are added, and the resulting suspension is heated under reflux for 4 hours. The suspension is then cooled to room temperature, poured into 100 ml of brine and extracted thrice with 30 ml each of *t*-butyl methyl ether. The combined organic phases are washed with 30 ml of brine, dried over Sodium sulfate, filtrated and evaporated *in vacuo*. 0.720 g of the title compound are obtained as a colorless oil crystallizing on standing; melting point 60-61°.

The starting material can be obtained as follows:

a) 2-[2-(3-Bromophenyl)-vinyl]-6-methyl-pyridine

A solution of 24 ml (200 mMol) of 2,6-dimethyl pyridine and 25.6 ml (207 mMol) of 3-bromobenzaldehyde in 38 ml of acetic anhydride is heated under reflux for 7.5 hours. The acetic anhydride is then evaporated *in vacuo*, and the residue is dissolved in 500 ml of 4N hydrochloric acid and twice extracted with 200 ml each of hexane. The water phase is then extracted four times with 300 ml each of tert.-butyl methyl ether. The combined organic phases are washed twice with 300 ml each of a saturated solution of $NaHCO_3$ in water, then once with 300 ml of brine (300 ml), dried over sodium sulfate, filtrated and evaporated *in vacuo* yielding 4.2 g of the title compound as colorless crystals of melting point 58-59°.

b) 2-[1,2-dibromo-2-(3-bromophenyl)-ethyl]-6-methyl-pyridine

1 g (3.6 mMol) of 2-(3-Bromo-phenylethynyl)-6-methyl-pyridine are dissolved in 5 ml of carbon tetrachloride, and the solution is heated to 55-60°. A solution of 0.23 ml (4.4 mMol) of bromine Br_2 in 1 ml of carbon tetrachloride is added dropwise. The reaction mixture is maintained at 55-60° for 30 minutes and then cooled to room temperature. The resulting precipitate is collected by filtration and dried *in vacuo*. 1.3 g of the title compound in form of yellow crystals of melting point 164-166are isolated.

EXAMPLE 6

3-[2-(6-Methylpyridin-2-yl)ethynyl]-benzonitrile

A mixture of 1 g (8.54 mMol) 2-ethynyl-6-methyl-pyridine (prepared in analogy to D. E. Ames et al., Synthesis, 1981, p. 364-5), 2.3 g (12.8 mMol) 3-bromo-benzonitrile, 0.47 g (0.7 mMol) bis-(triphenylphosphine)-palladium-II-chloride, 80 mg (0.41 mMol) cuprous iodide and 1.53 ml (15 mMol) triethylamine in 10 ml dimethylformamide is stirred for 3 hours at 90° C. The reaction mixture is cooled to ambient temperature, poured into water and extracted with dichloromethane. The organic layer is dried over sodium sulfate, filtered, evaporated to dryness and the residue is purified by chromatography on silica gel with hexane/ethyl acetate (4:1) as eluant. Crystallization from hexane of the obtained product affords 0.53 g (28.4 %) of the title compound as brown crystals, melting point 120-3° C.

EXAMPLE 7

In analogous manner to Example 1 (when X is alkenylene) or Example 5 (when X is alkynylene), the following compounds of formula I can be prepared:

Compound of formula I	**Melting point (°C)**
2-Styryl-pyridin-3-ol	249-252
2-Methyl-6-[2-(3-nitro-phenyl)-vinyl]-pyridine	100-101
2-[2-(2-Chloro-phenyl)-vinyl]-pyridine	colorless oil
2-Methyl-6-styryl-pyridine	40-42
Acetic acid 6-[2-(2-chloro-phenyl)-vinyl]-pyridin-3-yl ester	75-77
6-[2-(2-Chloro-phenyl)-vinyl]-pyridin-3-ol	168-171
Acetic acid 2-[2-(2-chloro-phenyl)-vinyl]-pyridin-3-yl ester	99-102

2-[2-(2-Chloro-phenyl)-vinyl]-pyridin-3-ol	232-234
6-Methyl-2-styryl-pyridin-3-ol	261 dec
Acetic acid 2-[2-(2-chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yl ester	92-94
2-[2-(2-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-ol	232-234
(Z)-6-Methyl-2-styryl-pyridin-3-ol	145-148
2-[2-(2-Chloro-phenyl)-vinyl]-6-methyl-pyridine	51-52
2-[2-(2-Fluoro-phenyl)-vinyl]-pyridine	69-70
2-[2-(2-Nitro-phenyl)-vinyl]-pyridine	97-99
Acetic acid 2-[2-(4-chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yl ester	102-103
Acetic acid 6-[2-(4-chloro-phenyl)-vinyl]-2-methyl-pyridin-3-yl ester	130-131
2-[2-(4-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-ol	275-278 dec
6-[2-(4-Chloro-phenyl)-vinyl]-2-methyl-pyridin-3-ol	265-270 dec
Acetic acid 6-methyl-2-[2-(2-nitro-phenyl)-vinyl]-pyridin-3-yl ester	139-140
6-Methyl-2-[2-(2-nitro-phenyl)-vinyl]-pyridin-3-ol	190-195 dec
Acetic acid 2-methyl-6-[2-(2-nitro-phenyl)-vinyl]-pyridin-3-yl ester	99-100
2-Methyl-6-[2-(2-nitro-phenyl)-vinyl]-pyridin-3-ol	230-233 dec
Acetic acid 2-[2-(3-chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yl ester	97-99
Acetic acid 6-[2-(3-chloro-phenyl)-vinyl]-2-methyl-pyridin-3-yl ester	112-114
2-[2-(3-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-ol	232-235
6-[2-(3-Chloro-phenyl)-vinyl]-2-methyl-pyridin-3-ol	230-232
(Z)-(6-Styryl-pyridin-2-yl)-methanol	69-70
(E)-(6-Styryl-pyridin-2-yl)-methanol	58-60
2,2'-(1,2-Ethenediyl)bis[6-methyl]-pyridine	108-110
Dimethyl-[3-(6-methyl-2-styryl-pyridin-3-yloxy)-propyl]-amine;hydrochloride salt	136-139
(E)-6-[2-(2-Pyridyl)vinyl]-2-picoline	56-57
2-Methyl-6-styryl-pyridine 1-oxide	102-103
2-Styryl-pyridine 1-oxide	156-159
(E)-6-Methyl-2-(2-pyridin-2-yl-vinyl)-pyridin-3-ol	240-242
(Z)-6-Methyl-2-(2-pyridin-2-yl-vinyl)-pyridin-3-ol; HCl salt	225-228
6-Styryl-pyridine-2-carbonitrile	92-93
2-[2-(2,6-Dichloro-phenyl)-vinyl]-6-methyl-pyridine	light yell. oil
3-Methoxy-6-methyl-2-styryl-pyridine	light yell. oil
6-Styryl-pyridine-2-carboxylic acid amide	141-142
2-[2-(6-Methyl-pyridin-2-yl)-vinyl]-benzonitrile	113-114

3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-benzonitrile	91-92
4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-benzonitrile	131-132
6-Styryl-pyridine-2-carboxylic acid; HCl Salt	209-212
6-Styryl-pyridine-2-carboxylic acid methyl ester	87-88
Acetic acid 2-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl ester	colorless oil
2-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenol	227-229
Acetic acid 2-methoxy-4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl ester	102-103
2-[2-(3-Chloro-phenyl)-vinyl]-6-methyl-pyridine	59-61
2-[2-(4-Chloro-phenyl)-vinyl]-6-methyl-pyridine	83-85
2-[2-(2-Chloro-phenyl)-vinyl]-5-ethyl-pyridine	34-35
1-(6-Styryl-pyridin-2-yl)-ethanone	67-68
6-[2-(2-Chloro-phenyl)-vinyl]-2-methyl-nicotinic acid ethyl ester	80-82
2-[2-(2-Chloro-phenyl)-vinyl]-6-methyl-nicotinic acid ethyl ester	70-72
2-[2-(6-Methyl-pyridin-2-yl)-vinyl]-benzoic acid; HCl salt	218-219
3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-benzoic acid	150-151
4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-benzoic acid	206-207
3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-benzoic acid methyl ester; HCl salt	237-238
4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-benzoic acid methyl ester	112-113
2-Methoxy-4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenol	116-119
{3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-methanol; HCl salt	230-231
6-Styryl-pyridine-2-carboxylic acid .tert.-butylamide	87-88
2-(2-Bromo-2-phenyl-vinyl)-6-methyl-pyridine; HCl salt	150-154
2-Methyl-6-phenylethynyl-pyridine; HCl salt	146-148
6-Styryl-pyridine-2-carboxylic acid hexylamide; HCl salt	118-125
6-[2-(2-Chloro-phenyl)-vinyl]-2-methyl-nicotinic acid	219-221 dec
2-[2-(2-Chloro-phenyl)-vinyl]-6-methyl-nicotinic acid	168-170
2-[2-(3,5-Dichloro-phenyl)-vinyl]-6-methyl-pyridine	75-77
2-Methyl-6-[2-(3-trifluoromethyl-phenyl)-vinyl]-pyridine	44-45
(E)-6-[2-(4-pyridyl)vinyl]-2-Picoline	72-73
N,N-Diethyl-3-[2-(6-methyl-pyridin-2-yl)-vinyl]-benzamide; HCl salt	227-228
N,N-Diethyl-4-[2-(6-methyl-pyridin-2-yl)-vinyl]-benzamide; HCl salt	183-184
(E)-6-[2-(3-pyridyl)vinyl]-2-Picoline	yellowish oil
{2-[2-(2-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-acetic acid ethyl ester	colorless gum

3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-.N.-(3-trifluoromethyl-phenyl)-benzamide; HCl salt	249-251
4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-.N.-(3-trifluoromethyl-phenyl)-benzamide	160-161
2-[2-(3-Nitro-phenyl)-vinyl]-pyridine	127-128
6-Styryl-pyridine-2-carboxylic acid (3-trifluoromethyl-phenyl)-amide	126-129
2-(6-Styryl-pyridin-2-yl)-propan-2-ol, HCl salt	171-174
2-Methyl-6-(2-thiophen-2-yl-vinyl)-pyridine, HCl salt	208-211
2-[2-(3-Chloro-phenyl)-vinyl]-pyridine	51-53
2-[2-(3-Cyano-phenyl)-vinyl]-pyridine	85-86
2-[2-(3-Bromo-phenyl)-vinyl]-6-methyl-pyridine	58-59
2-[2-(3-Bromo-phenyl)-2-fluoro-vinyl]-6-methyl-pyridine	58-59
2-[2-(3,5-Dimethylphenyl)-2-fluoro-vinyl]-6-methyl-pyridine	70-72
2-[2-(2,3-Dimethoxy-phenyl)-vinyl]-6-methyl-pyridine	colorless oil
2-[2-(2,3-Dichloro-phenyl)-vinyl]-6-methyl-pyridine	67-68
2-[2-(3-Chloro-phenyl)-1-methyl-vinyl]-pyridine	colorless oil
{2-[2-(2-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yl}-methanol	87-90
2-Methyl-6-[2-(3-trimethylsilanylethynyl-phenyl)-vinyl]-pyridine	yellowish oil
2-[2-(3,4-Difluoro-phenyl)-vinyl]-6-methyl-pyridine	61-62
2-[2-(3-Ethynyl-phenyl)-vinyl]-6-methyl-pyridine	yellowish oil
2-[2-(3,5-Difluoro-phenyl)-vinyl]-6-methyl-pyridine	yellowish oil
2-[2-(3-Fluoro-phenyl)-vinyl]-6-methyl-pyridine	yellowish oil
2-[2-(3-Methoxy-phenyl)-vinyl]-6-methyl-pyridine	yellowish oil
2-Methyl-6-[2-(3-phenoxy-phenyl)-vinyl]-pyridine	yellowish oil
2-[2-(3-Benzyloxy-phenyl)-vinyl]-6-methyl-pyridine	68-69
2-[2-(2,5-Difluoro-phenyl)-vinyl]-6-methyl-pyridine	44-45
{2-[2-(2-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-acetic acid	230-233
(3-{2-[2-(3-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethyl-amine	203-205
{6-[2-(2-Chloro-phenyl)-vinyl]-2-methyl-pyridin-3-yl}-methanol	131-133
2-(3-Bromo-phenylethynyl)-6-methyl-pyridine	61-63
2-Methyl-6-{2-[3-(3-trifluoromethyl-phenoxy)-phenyl]-vinyl}-pyridine	yellowish oil
2-[2-(3,5-Dimethoxy-phenyl)-vinyl]-6-methyl-pyridine	43-45
2-[2-(3-Chloro-phenyl)-vinyl]-3-methoxy-6-methyl-pyridine	52-53
Acetic acid 4-bromo-2-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl ester	yellowish oil
Acetic acid 3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl ester	yellowish oil

2-[2-(3,4-Dichloro-phenyl)-vinyl]-6-methyl-pyridine	73-75
4-Bromo-2-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenol	246-248
Acetic acid 2-[2-(3,5-dichloro-phenyl)-vinyl]-6-methyl-pyridin-3-yl ester	156-158
Acetic acid 6-[2-(3,5-dichloro-phenyl)-vinyl]-2-methyl-pyridin-3-yl ester	159-161
Acetic acid 2-[2-(3,5-dichloro-phenyl)-vinyl]-pyridin-3-yl ester	154-156
2-Methyl-6-(2-naphthalen-1-yl-vinyl)-pyridine	yellowish oil
2-[2-(2,3-Dihydro-benzo[1,4]dioxin-6-yl)-vinyl]-6-methyl-pyridine	99-101
2-Methyl-6-(2-naphthalen-2-yl-vinyl)-pyridine	97-99
2-Methyl-6-(2-m-tolyl-vinyl)-pyridine	yellowish oil
2-{2-[3-(3,5-Dichloro-phenoxy)-phenyl]-vinyl}-6-methyl-pyridine	yellowish gum
2-[2-(3-Chloro-phenyl)-propenyl]-6-methyl-pyridine	yellowish oil
2-[2-(2,3-Dihydro-benzofuran-5-yl)-vinyl]-6-methyl-pyridine	88-90
2-[2-(4-Fluoro-phenyl)-vinyl]-6-methyl-pyridine	50-51
2-Methyl-6-(2-o-tolyl-vinyl)-pyridine	yellowish oil
2-Methyl-6-(2-p-tolyl-vinyl)-pyridine	85-86
2-Methyl-6-(2-p-tolyl-propenyl)-pyridine	yellowish oil
3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenylamine	126-129
(2,3-Dimethoxy-7-nitro-quinoxalin-5-ylmethyl)-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-amine	pale orange foam
N-{3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-acetamide	147
N-{3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-2-phenyl-acetamide	156
2,2-Dimethyl-N-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-propionamide	166-168
Thiophene-2-carboxylic acid {3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-amide	197 dec
Cyclohexanecarboxylic acid {3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-amide	215
1-(4-Bromo-phenyl)-3-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-urea	197 dec
2-Methyl-6-[2-(4-nitro-phenyl)-vinyl]-pyridine	134-135
4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenylamine	147-148
2-[2-(3,5-Dichloro-phenyl)-vinyl]-6-methyl-pyridin-3-ol	218-220
6-[2-(3,5-Dichloro-phenyl)-vinyl]-2-methyl-pyridin-3-ol	286 dec
2-[2-(3,5-Dichloro-phenyl)-vinyl]-pyridin-3-ol	240-242
2-[2-(6-Chloro-benzo[1,3]dioxol-5-yl)-vinyl]-6-methyl-pyridine	131-132
2-[2-(2,3-Difluoro-phenyl)-vinyl]-6-methyl-pyridine	55-56
2-[2-(3,4-Dichloro-phenyl)-propenyl]-6-methyl-pyridine	yellowish oil

2-[2-(3,5-Bis-trifluoromethyl-phenyl)-vinyl]-6-methyl-pyridine	85-86
Acetic acid 2-methoxy-6-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl ester	yellowish oil
2-Methoxy-6-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenol	118-120
2-Methyl-6-[2-(2,3,6-trifluoro-phenyl)-vinyl]-pyridine	59-62
2-[2-(4-Fluoro-3-trifluoromethyl-phenyl)-vinyl]-6-methyl-pyridine	yellowish oil
2-Methyl-6-(2,3,6-trifluoro-phenylethynyl)-pyridine	93-94
Acetic acid 4-chloro-2-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl ester	yellowish oil
Acetic acid 2,6-di-.tert.-butyl-4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl ester	127-128
3-(6-Methyl-pyridin-2-ylethynyl)-benzamide	187-189
Acetic acid 4-bromo-2-methoxy-6-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl ester	151-153
2-(6-Chloro-benzo[1,3]dioxol-5-ylethynyl)-6-methyl-pyridine	105-106 light brown crystals
2-[2-(3,5-Dichloro-phenyl)-vinyl]-3-methoxy-6-methyl-pyridine	127-129
2-[2-(3,5-Dichloro-phenyl)-vinyl]-3-methoxy-pyridine	111-113
5-Azido-2-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenol	143 dec
2-[2-(Pyridin-3-yl)ethynyl]-6-methyl-pyridine	light yellow crystals 60-61
N-{3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-succinamic acid	212-213
1-tert.-Butyl-3-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-urea	191-192
5-({3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenylamino}-methyl)-7-nitro-1,4-dihydro-quinoxaline-2,3-dione	250 dec
Tetrahydro-furan-2-carboxylic acid {3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-amide	160-161
(1-{3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenylcarbamoyl}-2-phenyl-ethyl)-carbamic acid tert.-butyl ester	colorless foam
({3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenylcarbamoyl}-methyl)-carbamic acid tert.-butyl ester	colorless foam
Diethyl-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-amine	217 dec
Ethyl-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-amine	225 dec
Ethyl-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-amine	183 dec
2-(2-Ethoxy-3,6-difluoro-phenylethynyl)-6-methyl-pyridine	yellowish oil
2-(3,5-Difluoro-phenylethynyl)-6-methyl-pyridine	yellowish oil
2-(3-Fluoro-phenylethynyl)-6-methyl-pyridine	26-28
2-[2-(3,5-Dimethyl-phenyl)-vinyl]-6-methyl-pyridine	56-57

2-[2-(3,4-Dimethoxy-phenyl)-vinyl]-6-methyl-pyridine	55-56
2-(3,4-Dichloro-phenylethynyl)-6-methyl-pyridine	73-74
2-(4-Ethoxy-3-trifluoromethyl-phenylethynyl)-6-methyl-pyridine	61-62
2-(4-Fluoro-phenylethynyl)-6-methyl-pyridine	98-100
2-Methyl-6-.o.-tolylethynyl-pyridine	yellowish oil
2-(3,4-Difluoro-phenylethynyl)-6-methyl-pyridine	65-68
2-Methyl-6-[2-(2,3,5-trichloro-phenyl)-vinyl]-pyridine	80-82
1-[3-(6-Methyl-pyridin-2-ylethynyl)-phenyl]-ethanone	76-78
2-Methyl-6-(3-trifluoromethyl-phenylethynyl)-pyridine	35-37
2-Methyl-6-(3-nitro-phenylethynyl)-pyridine	99.5-102.5
6-[2-(3,5-Dichloro-phenyl)-vinyl]-3-methoxy-2-methyl-pyridine	98-100
{2-[2-(2-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yl}-morpholin-4-yl-methanone	123-125
(3-{2-[2-(3,5-Dichloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethyl-amine hydrochloride salt	207-210
N-{4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-succinamic acid	201 dec
N-{4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-2-phenyl-acetamide	236-237 dec
({4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenylcarbamoyl}-methyl)-carbamic acid .tert.-butyl ester	144-145 dec
1-tert.-Butyl-3-{4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-urea	209 dec
{3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-thiophen-2-ylmethyl-amine hydrochloride salt	161-162
Cyclohexylmethyl-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-amine hydrochloride salt	178-179 dec
{4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-thiophen-2-ylmethyl-amine	100
Cyclohexylmethyl-{4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-amine	106-107
2-Amino-N-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-3-phenyl-propionamide	102
2-Amino-N-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-acetamide	105
2-Amino-N-{4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-acetamide	217-219 dec
1-[1-({2-[2-(2-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-acetyl)-piperidin-4-yl]-imidazolidin-2-one	amorphous foam
(1-{4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenylamino}-ethyl)-phosphonic acid dimethyl ester	orange amorphous solid
2-[2-(2-Methoxy-phenyl)-vinyl]-6-methyl-pyridine	129-130

2-(3-Ethoxy-4-fluoro-phenylethynyl)-6-methyl-pyridine	82-83
2-(3-Chloro-phenylethynyl)-6-methyl-pyridine	57-59
1-(3-Pyridin-2-ylethynyl-phenyl)-ethanone	48-51
4-Chloro-2-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenol	256-260
4-Bromo-2-methoxy-6-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenol	121-123
2-Methyl-6-.m.-tolylethynyl-pyridine	57-58
2-(2,5-Difluoro-phenylethynyl)-6-methyl-pyridine	49-50
2-(3,5-Dimethyl-phenylethynyl)-6-methyl-pyridine	yellowish oil
2-[2-(3,5-Dibromo-phenyl)-vinyl]-6-methyl-pyridine	68-70
2-Methyl-6-[2-(pyrimidin-5-yl)-ethynyl]-pyridine	110-112
(2-{2-[2-(3-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-ethyl)-dimethyl-amine	165-167
Acetic acid 1-{4-[2-(6-methyl-pyridin-2-yi)-vinyl]-phenyl}-ethyl ester	
3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenol	250-251
3-(6-Methyl-pyridin-2-ylethynyl)-phenylamine	129-130
N-[3-(6-Methyl-pyridin-2-ylethynyl)-phenyl]-2-phenyl-acetamide	133-135 dec
Thiophene-2-carboxylic acid [3-(6-methyl-pyridin-2-ylethynyl)-phenyl]-amide	156-157 dec
2-Methyl-6-(thiophen-2-ylethynyl)-pyridine	34-36
3-(6-Methyl-pyridin-2-ylethynyl)-benzoic acid ethyl ester	56-58
2-(3,5-Dibromo-phenylethynyl)-6-methyl-pyridine	100:101
{2-[2-(2-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-ylmethyl}-dimethyl-amine	227-229 dec
(3-{6-[2-(3-Chloro-phenyl)-vinyl]-2-methyl-pyridin-3-yloxy}-propyl)-dimethyl-	184-186
5-Azido-4-iodo-2-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenol	red glass
2,6-Di-tert-butyl-4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenol	126-127
1-{4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-ethanol	97-99
2-Methyl-6-[2-(pyrimidin-2-yl)-ethynyl]-pyridine	144-145
[3-(6-Methyl-pyridin-2-ylethynyl)-phenyl]-phenyl-methanone	99-100
6-(6-Methyl-pyridin-2-ylethynyl)-3,4-dihydro-1H-quinolin-2-one	189-191
2-(3-{2-[2-(3-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-isoindole-1,3-dione	101-103
3-Methoxy-6-methyl-2-.m.-tolylethynyl-pyridine	brown oil
Acetic acid 2-[2-(6-methyl-pyridin-2-yl)-vinyl]-4-nitro-phenyl ester	129-131
6-(6-Methyl-pyridin-2-ylethynyl)-indan-1-one	160-165
2-Methyl-6-[2-(pyrazin-2-yl)-ethynyl]-pyridine	95-96

N-Methyl-N-(3-{4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenoxy}-propyl)-acetamide	62-70
2-[2-(3,5-Bis-trifluoromethyl-phenyl)-1-ethoxy-vinyl]-6-methyl-pyridine	yellow oil
Acetic acid 2-phenylethynyl-pyridin-3-yl ester	brown oil
Acetic acid 6-methyl-2-.m.-tolylethynyl-pyridin-3-yl ester	brown oil
Acetic acid 4-[2-(6-methyl-pyridin-2-yl)-vinyl]-2-nitro-phenyl ester	91-93
2-[2-(6-Methyl-pyridin-2-yl)-vinyl]-4-nitro-phenol	275 dec
Dimethyl-[3-(2-phenylethynyl-pyridin-3-yloxy)-propyl]-amine	yellowish oil
Dimethyl-(3-{4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenoxy}-propyl)-amine	240-243
1-{4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-ethanone	56-58
2-(3-Fluoro-phenylethynyl)-quinoline	81-83
Acetic acid 2-methyl-6-styryl-pyridin-3-yl ester	93-96
4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-2-nitro-phenol	141-143
3-Ethoxy-4-[2-(6-methyl-pyridin-2-yl)-vinyl]-2-nitro-phenol	175-178 dec
4-(6-Methyl-pyridin-2-ylethynyl)-2-nitro-phenol	184-187 dec
Acetic acid 2-[2-(6-methyl-pyridin-2-yl)-vinyl]-6-nitro-phenyl ester	105-110 dec
Dimethyl-[3-(6-methyl-2-phenylethynyl-pyridin-3-yloxy)-propyl]-amine	yellow gum
2-Azido-4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenol	155-157 dec
Dimethyl-[3-(6-methyl-2-.m.-tolylethynyl-pyridin-3-yloxy)-propyl]-amine	yellowish oil
2-(3-Methanesulfonyl-phenylethynyl)-6-methyl-pyridine	108-110 dec
3-{2-[2-(3-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propylamine	186-189
4-Azido-.N.-(3-{2-[2-(3-chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-2-hydroxy-benzamide	99-102 dec
3-[3-(3-Dimethylamino-propoxy)-6-methyl-pyridin-2-ylethynyl]-benzonitrile	yellow gum
5-(6-Methyl-pyridin-2-ylethynyl)-indan-1-one	133-134
2-Methyl-6-(2,3,5-trichloro-phenylethynyl)-pyridine	112-114
2-[2-(6-methyl-pyridin-3-yl)ethynyl]-6-methyl-pyridine	118-119
Dimethyl-{3-[6-methyl-2-(3-trifluoromethyl-phenylethynyl)-pyridin-3-yloxy]-propyl}-amine	yellow gum
2-[2-(6-methyl-pyridin-3-yl)ethynyl]-3-methoxy 6-methyl-pyridine hydrcchloride salt	198-199
2-Methyl-6-(5,6,7,8-tetrahydro-naphthalen-2-ylethynyl)-pyridine	50-51
3-[2-(3-Chloro-phenylethynyl)-6-methyl-pyridin-3-yloxy]-propylamine	151-153
(3-{4-Bromo-2-methoxy-6-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenoxy}-propyl)-dimethyl-amine;	211-215

[6-(3-Fluoro-phenylethynyl)-pyridin-2-yl]-dimethyl-amine	brown oil
6'-(3-Fluoro-phenylethynyl)-3,4,5,6-tetrahydro-2.H.-[1,2']bipyridinyl	brown gum
{3-[2-(3-Chloro-phenylethynyl)-6-methyl-pyridin-3-yloxy]-propyl}-dimethyl-amine	158-160
4-Azido-.N.-{3-[2-(3-chloro-phenylethynyl)-6-methyl-pyridin-3-yloxy]-propyl}-2-hydroxy-benzamide	161-163 dec
1-[3-(6-Methyl-pyridin-2-ylethynyl)-phenyl]-1H-[1,2,4]triazole-3-carboxylic acid ethyl ester	105-110 dec
1-[3-(6-Methyl-2-phenylethynyl-pyridin-3-yloxy)-propyl]-piperidin-3-ol	108-109
2-Ethynyl-6-(3-fluoro-phenylethynyl)-pyridine	89-90
3-Methyl-6-(6-methyl-pyridin-2-ylethynyl)-3H-benzooxazol-2-one	172-174
1-[3-(6-Methyl-pyridin-2-ylethynyl)-phenyl]-1H-[1,2,4]triazole-3-carboxylic acid dimethylamide	154-157
1-[3-(6-Methyl-2-phenylethynyl-pyridin-3-yloxy)-propyl]-piperidin-4-ol	amorphous white solid
5-(6-Methyl-pyridin-2-ylethynyl)-2-nitro-phenol	150-151 dec
5-[2-Bromo-2-(6-methyl-pyridin-2-yl)-vinyl]-2-nitro-phenol	158-159
5-[2-(6-Methyl-pyridin-2-yl)-E-vinyl]-2-nitro-phenol	171-173
5-[2-(6-Methyl-pyridin-2-yl)-Z-vinyl]-2-nitro-phenol	108-110
4-Azido-2-hydroxy-.N.-[3-(6-methyl-pyridin-2-ylethynyl)-phenyl]-benzamide	180-182 dec
5-(3-Dimethylamino-propoxy)-6-phenylethynyl-pyridine-2-carboxylic acid ethyl ester	160-162
6-Methyl-2-styryl-pyrimidin-4-ol	221-225
2-Ethyl-6-(3-fluoro-phenylethynyl)-pyridine	brown oil
2-(3,5-Dichloro-phenylethynyl)-6-methyl-pyridine	74-76
2-Methyl-6-(3-trifluoromethoxy-phenylethynyl)-pyridine	<30; brown crystals
2-Methyl-6-(3-[1,2,4]triazol-1-yl-phenylethynyl)-pyridine	128-130
4-(6-Methyl-pyridin-2-ylethynyl)-phthalonitrile	138-140
2-Methyl-6-{2-[3-(1.H.-tetrazol-5-yl)-phenyl]-vinyl}-pyridine; compound with formic acid	234-240
3-[2-(3,5-Dichloro-phenylethynyl)-6-methyl-pyridin-3-yloxy]-propylamine	97-100
{3-[2-(3,5-Dichloro-phenylethynyl)-6-methyl-pyridin-3-yloxy]-propyl}-dimethyl-amine	171-173
2-(3,5-Dimethyl-phenylethynyl)-3-methoxy-6-methyl-pyridine	yellowish oil
2-[2-(3-Fluoro-phenyl)-vinyl]-6-methyl-pyridin-3-ol	251-253 Dec.

6-(3-Fluoro-phenylethynyl)-2-methyl-nicotinic acid ethyl ester	84-86
2-Azido-5-(6-methyl-pyridin-2-ylethynyl)-phenol	153-155 dec
6-(3,4-Dimethoxy-phenylethynyl)-5-(3-dimethylamino-propoxy)-pyridine-2-carboxylic acid ethyl ester	149-152
2-(4-Methoxy-3-trifluoromethyl-phenylethynyl)-6-methyl-pyridine	56-87
2-(3-Fluoro-phenylethynyl)-6-methoxy-pyridine	brown oil
2-(3-Fluoro-phenylethynyl)-5-methyl-pyridine	74-76
6-(3,5-Dichloro-phenylethynyl)-5-(3-dimethylamino-propoxy)-pyridine-2-carboxylic acid ethyl ester	195-198
5-(3-Dimethylamino-propoxy)-6-(3,5-dimethyl-phenylethynyl)-pyridine-2-carboxylic acid ethyl ester	187-190
6-(3-Fluoro-phenylethynyl)-2-methyl-nicotinic acid	173-175
[6-(3-Fluoro-phenylethynyl)-2-methyl-pyridin-3-yl]-methanol	116-118
[4-(4-Fluoro-benzoyl)-piperidin-1-yl]-[6-(3-fluoro-phenylethynyl)-2-methyl-pyridin-3-yl]-methanone	138-140
2-(3-Fluoro-phenylethynyl)-6-methyl-nicotinic acid ethyl ester	brown oil
2-(3-Fluoro-phenylethynyl)-4,6-dimethyl-pyridine	brown oil
6-(3-Fluoro-phenylethynyl)-.N.-(5-methoxy-indan-2-ylmethyl)-2-methyl-nicotinamide	157-159
{[6-(3-Fluoro-phenylethynyl)-2-methyl-pyridine-3-carbonyl]-amino}-phenyl-acetic acid methyl ester	133-135
2-Methyl-6-(5-methyl-thiophen-2-ylethynyl)-pyridine	58-59
2-Methyl-6-(2,3,5-trimethyl-phenylethynyl)-pyridine	brown oil
3-{2-[2-(3-Fluoro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propan-1-ol	86-88
[6-(3-Fluoro-phenylethynyl)-2-methyl-pyridin-3-ylmethyl]-dimethyl-amine	220-222
2,2-Dimethyl-propionic acid 3-[2-(3-fluoro-phenylethynyl)-6-methyl-pyridin-3-yloxy]-propyl ester	yellowish oil
2-Azido-4-iodo-5-(6-methyl-pyridin-2-ylethynyl)-phenol	140 dec
6-Azido-2,4-diiodo-3-(6-methyl-pyridin-2-ylethynyl)-phenol	162 dec
4-Azido-2-hydroxy-5-iodo-.N.-[3-(6-methyl-pyridin-2-ylethynyl)-phenyl]-benzamide	185 dec
Acetic acid 3-acetoxymethyl-5-(6-methyl-pyridin-2-ylethynyl)-benzyl ester	brown oil
(Benzyl-{[2-(3-fluoro-phenylethynyl)-6-methyl-pyridin-3-yloxy]-acetyl}-amino)-acetic acid ethyl ester	brown oil
2-[2-(3-Fluoro-phenyl)-vinyl]-6-methyl-isonicotinic acid ethyl ester	76-77

3-[2-(3-Fluoro-phenylethynyl)-6-methyl-pyridin-3-yloxy]-propan-1-ol	72-74
[3-Hydroxymethyl-5-(6-methyl-pyridin-2-ylethynyl)-phenyl]-methanol	115-117
(3-{2-[2-(3,5-Dimethyl-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethyl-amine	yellowish gum
[4-(4-Fluoro-benzoyl)-piperidin-1-yl]-{6-[2-(3-fluoro-phenyl)-vinyl]-2-methyl-pyridin-3-yl}-methanone	156-158
2-[2-(3-Fluoro-phenyl)-vinyl]-6-methyl-isonicotinic acid	245-248
{6-[2-(2-Chloro-phenyl)-vinyl]-2-methyl-pyridin-3-yl}-[4-(4-fluoro-benzoyl)-piperidin-1-yl]-methanone	109-112
2-(3-Ethynyl-phenylethynyl)-6-methyl-pyridine	48-49
(3-{2-[2-(2,6-Dichloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethyl-amine hydrochloride salt	207-210
(3-{2-[2-(2,3-Dichloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethyl-amine hydrochloride salt	161-169
4-[6-(3-Fluoro-phenylethynyl)-2-methyl-pyridine-3-carbonyl]-piperazine-1-carboxylic acid .tert.-butyl ester	97-99
[6-(3-Fluoro-phenylethynyl)-2-methyl-pyridin-3-yl]-piperazin-1-yl-methanone	250-252 dec
[4-(4-Azido-2-hydroxy-benzoyl)-piperazin-1-yl]-[6-(3-fluoro-phenylethynyl)-2-methyl-pyridin-3-yl]-methanone	186-188 dec
(3-{2-[2-(2,4-Dichloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethyl-amine hydrochloride salt	170-176
2-(3-Fluoro-phenylethynyl)-6-methyl-isonicotinic acid ethyl ester	89-91
2-(3-Fluoro-phenylethynyl)-6-methyl-isonicotinic acid .tert.-butyl ester	94-96
2-(3-Fluoro-phenylethynyl)-6-methyl-isonicotinic acid	231 dec
[2-(3-Fluoro-phenylethynyl)-6-methyl-pyridin-4-yl]-methanol	143-146
[4-(4-Fluoro-benzoyl)-piperidin-1-yl]-[2-(3-fluoro-phenylethynyl)-6-methyl-pyridin-4-yl]-methanone	156-158
3-Allyloxy-2-[2-(3,5-dichloro-phenyl)-vinyl]-6-methyl-pyridine	105-106
[2-(3-Fluoro-phenylethynyl)-6-methyl-pyridin-4-yl]-morpholin-4-yl-methanone	114-116
Acetic acid 3-(6-methyl-pyridin-2-ylethynyl)-benzyl ester	brown oil
[2-(3-Fluoro-phenylethynyl)-6-methyl-pyridin-4-ylmethyl]-dimethyl-amine	209-212
(3-{2-[2-(3,5-Dichloro-phenyl)-propenyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethyl-amine hydrochloride salt	182-184
2-(3-Fluoro-phenylethynyl)-3-methoxy-6-methyl-pyridine	yellowish oil

(3-{2-[2-(3,5-Dichloro-phenyl)-vinyl]-pyridin-3-yloxy}-propyl)-dimethyl-amine hydrochloride salt	171-174
(4-Azido-2-hydroxy-5-iodo-phenyl)-{4-[6-(3-fluoro-phenylethynyl)-2-methyl-pyridine-3-carbonyl]-piperazin-1-yl}-methanone	195-200 dec
4-Azido-.N.-{3-[2-(3-chloro-phenylethynyl)-6-methyl-pyridin-3-yloxy]-propyl}-2-hydroxy-5-iodo-benzamide	142-150 dec
4-(2-Pyridin-2-yl-vinyl)-benzoic acid ethyl ester	100-102
(3-{2-[2-(4-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethyl-amine hydrochloride salt	159-171
[3-(6-Methyl-pyridin-2-ylethynyl)-phenyl]-methanol	43-45
6-(3-Fluoro-phenylethynyl)-nicotinic acid .tert.-butyl ester	96-98
(3-{2-[2-(3,4-Dichloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethyl-amine hydrochloride salt	174-177
2-(1-Bromo-2-phenyl-vinyl)-4-methyl-pyrimidine	yellow oil
6-(3-Fluoro-phenylethynyl)-nicotinic acid	223 dec.
[4-(4-Fluoro-benzoyl)-piperidin-1-yl]-[6-(3-fluoro-phenylethynyl)-pyridin-3-yl]-methanone	136.0-139.0
2-(2-.tert.-Butoxy-3,6-difluoro-phenylethynyl)-6-methyl-pyridine	72.0-74.0
2-Methyl-6-[2-(2,4,5-trifluoro-phenyl)-vinyl]-pyridine	74-76
2-Methyl-6-[2-(2,3,4-trifluoro-phenyl)-vinyl]-pyridine	79-82
3-(6-Methyl-pyridin-2-ylethynyl)-phenol	142-144
2-Methyl-6-[2-(3,4,5-trifluoro-phenyl)-vinyl]-pyridine	74-76
2-(3-Methoxy-phenylethynyl)-6-methyl-pyridine	55-57
2-Methyl-6-(2,3,4-trifluoro-phenylethynyl)-pyridine	104-106

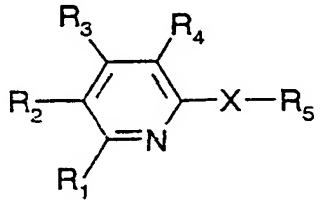
(dec = decomposition)

Claims:

1. A 2-arylalkenyl- , 2-heteroarylalkenyl-, 2-arylalkynyl-, 2-heteroarylalkynyl-, 2-arylazo- and 2-heteroarylazo- pyridine or a pharmaceutically acceptable salt thereof, for use in the treatment of disorders associated with irregularities of the glutamatergic signal transmission, and of nervous system disorders mediated full or in part by mGluR5.

2. A 2-arylalkenyl- , 2-heteroarylalkenyl-, 2-arylalkynyl-, 2-heteroarylalkynyl-, 2-arylazo- and 2-heteroarylazo- pyridine or a pharmaceutically acceptable salt thereof, for use in the treatment of epilepsy, cerebral ischemia, ischemic diseases of the eye, muscle spasms, convulsions, pain, acute, traumatic and chronic degenerative processes of the nervous system and psychiatric diseases.

3. A compound of formula I



(I),

wherein

R_1 denotes hydrogen, lower alkyl,hydroxy-lower alkyl, lower alkyl-amino, piperidino, carboxy, esterified carboxy, amidated carboxy, unsubstituted or lower alkyl-, lower alkoxy-, halo- and/or trifluoromethyl-substituted N-lower-alkyl-N-phenylcarbamoyl, lower alkoxy, halo-lower alkyl or halo-lower alkoxy,

R_2 denotes hydrogen, lower alkyl, carboxy, esterified carboxy, amidated carboxy, hydroxy-lower alkyl, hydroxy, lower alkoxy or lower alkanoyloxy, 4-(4-fluoro-benzoyl)-piperidin-1-yl-carboxy, 4-t.-butyloxycarbonyl-piperazin-1-yl-carboxy, 4-(4-azido-2-hydroxybenzoyl)-piperazin-1-yl-carboxy or 4-(4-azido-2-hydroxy-3-iodo-benzoyl)-piperazin-1-yl-carboxy,

R_3 represents hydrogen, lower alkyl, carboxy, lower alkoxy-carbonyl, lower alkyl-carbamoyl, hydroxy- lower alkyl, di- lower alkyl- aminomethyl, morpholinocarbonyl or 4-(4-fluoro-benzoyl)-piperidin-1-yl-carboxy,

R_4 represents hydrogen, lower alkyl, hydroxy, hydroxy-lower alkyl, amino-lower alkyl, lower alkylamino-lower alkyl, di-lower alkylamino-lower alkyl, unsubstituted or hydroxy-substituted lower alkyleneamino-lower alkyl, lower alkoxy, lower alkanoyloxy, amino-lower alkoxy, lower alkylamino-lower alkoxy, di-lower alkylamino-lower alkoxy,

phthalimido-lower alkoxy, unsubstituted or hydroxy- or 2-oxo-imidazolidin-1-yl-substituted lower alkyleneamino-lower alkoxy, carboxy, esterified or amidated carboxy, carboxy-lower-alkoxy or esterified carboxy-lower-alkoxy,
X represents an optionally halo-substituted lower alkenylene or alkynylene group bonded via vicinal unsaturated carbon atoms or an azo (-N=N-) group, and
R_5 denotes an aromatic or heteroaromatic group which is unsubstituted or substituted by one or more substituents selected from lower alkyl, halo, halo-lower alkyl, halo-lower alkoxy, lower alkenyl, lower alkynyl, unsubstituted or lower alkyl-, lower alkoxy-, halo- and/or trifluoromethyl-substituted phenyl, unsubstituted or lower alkyl-, lower alkoxy-, halo- and/or trifluoromethyl-substituted phenyl-lower alkynyl, hydroxy, hydroxy-lower alkyl, lower alkanoyloxy-lower alkyl, lower alkoxy, lower alkenyloxy, lower alkylenedioxy, lower alkanoyloxy, amino-, lower alkylamino-, lower alkanoylamino- or N-lower alkyl-N-lower alkanoylamino-lower alkoxy, unsubstituted or lower alkyl- lower alkoxy-, halo- and/or trifluoromethyl-substituted phenoxy, unsubstituted or lower alkyl- lower alkoxy-, halo- and/or trifluoromethyl-substituted phenyl-lower alkoxy, acyl, carboxy, esterified carboxy, amidated carboxy, cyano, carboxy-lower alkylamino, esterified carboxy-lower alkylamino, amidated carboxy-lower alkylamino, phosphono-lower alkylamino, esterified phosphono-lower alkylamino, nitro, amino, lower alkylamino, di-lower alkylamino, acylamino, N-acyl-N-lower alkylamino, phenylamino, phenyl-lower alkylamino, cycloalkyl-lower alkylamino or heteroaryl-lower alkylamino each of which may be unsubstituted or lower alkyl-lower alkoxy-, halo- and/or trifluoromethyl-substituted,
in free form or in form of a photoaffinity ligand, a radioactive marker, an N-oxide or a pharmaceutically acceptable salt,

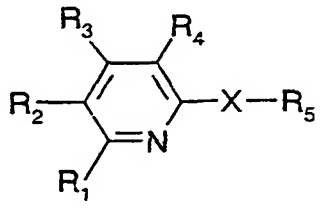
for use in the treatment of disorders associated with irregularities of the glutaminergic signal transmission, and of nervous system disorders mediated full or in part by mGluR5.

4. The use of a compound according to claim 3, in the treatment of disorders associated with irregularities of the glutamatergic signal transmission, and of nervous system disorders mediated full or in part by mGluR5.

5. The use of a compound according to claim 3, for the manufacture of a pharmaceutical composition designed for the treatment of disorders associated with irregularities of

the glutamatergic signal transmission, and of nervous system disorders mediated full or in part by mGluR5.

6. A compound of formula I



(I),

wherein

R_1 denotes hydrogen, lower alkyl, hydroxy-lower alkyl, lower alkyl-amino, piperidino, carboxy, esterified carboxy, amidated carboxy, unsubstituted or lower alkyl-, lower alkoxy-, halo- and/or trifluoromethyl-substituted N-lower-alkyl-N-phenylcarbamoyl, lower alkoxy, halo-lower alkyl or halo-lower alkoxy,

R_2 denotes hydrogen, lower alkyl, carboxy, esterified carboxy, amidated carboxy, hydroxy-lower alkyl, hydroxy, lower alkoxy or lower alkanoyloxy, 4-(4-fluoro-benzoyl)-piperidin-1-yl-carboxy, 4-t.-butyloxycarbonyl-piperazin-1-yl-carboxy, 4-(4-azido-2-hydroxybenzoyl)-piperazin-1-yl-carboxy or 4-(4-azido-2-hydroxy-3-iodo-benzoyl)-piperazin-1-yl-carboxy,

R_3 represents hydrogen, lower alkyl, carboxy, lower alkoxy-carbonyl, lower alkyl-carbamoyl, hydroxy- lower alkyl, di- lower alkyl- aminomethyl, morpholinocarbonyl or 4-(4-fluoro-benzoyl)-piperidin-1-yl-carboxy,

R_4 represents hydrogen, lower alkyl, hydroxy, hydroxy-lower alkyl, amino-lower alkyl, lower alkylamino-lower alkyl, di-lower alkylamino-lower alkyl, unsubstituted or hydroxy-substituted lower alkyleneamino-lower alkyl, lower alkoxy, lower alkanoyloxy, amino-lower alkoxy, lower alkylamino-lower alkoxy, di-lower alkylamino-lower alkoxy, phthalimido-lower alkoxy, unsubstituted or hydroxy- or 2-oxo-imidazolidin-1-yl-substituted lower alkyleneamino-lower alkoxy, carboxy, esterified or amidated carboxy, carboxy-lower-alkoxy or esterified carboxy-lower-alkoxy,

X represents an optionally halo-substituted lower alkenylene or alkynylene group bonded via vicinal unsaturated carbon atoms or an azo (-N=N-) group, and

R_5 denotes an aromatic or heteroaromatic group which is unsubstituted or substituted by one or more substituents selected from lower alkyl, halo, halo-lower alkyl, halo-lower alkoxy, lower alkenyl, lower alkynyl, unsubstituted or lower alkyl-, lower alkoxy-, halo- and/or trifluoromethyl-substituted phenyl, unsubstituted or lower alkyl-, lower alkoxy-, halo- and/or trifluoromethyl-substituted phenyl-lower alkynyl, hydroxy,

hydroxy-lower alkyl, lower alkanoyloxy-lower alkyl, lower alkoxy, lower alkenyloxy, lower alkylenedioxy, lower alkanoyloxy, amino-, lower alkylamino-, lower alkanoylamino- or N-lower alkyl-N-lower alkanoylamino-lower alkoxy, unsubstituted or lower alkyl- lower alkoxy-, halo- and/or trifluoromethyl-substituted phenoxy, unsubstituted or lower alkyl- lower alkoxy-, halo- and/or trifluoromethyl-substituted phenyl-lower alkoxy, acyl, carboxy, esterified carboxy, amidated carboxy, cyano, carboxy-lower alkylamino, esterified carboxy-lower alkylamino, amidated carboxy-lower alkylamino, phosphono-lower alkylamino, esterified phosphono-lower alkylamino, nitro, amino, lower alkylamino, di-lower alkylamino, acylamino, N-acyl-N-lower alkylamino, phenylamino, phenyl-lower alkylamino, cycloalkyl-lower alkylamino or heteroaryl-lower alkylamino each of which may be unsubstituted or lower alkyl-lower alkoxy-, halo- and/or trifluoromethyl-substituted,
in free form or in form of a photoaffinity ligand, a radioactive marker, an N-oxide or a pharmaceutically acceptable salt,
provided that, when R_3 is hydrogen,
a) in compounds of the formula I in which R_1, R_2 and R_4 are hydrogen, R_5 is different from phenyl, monohalophenyl, 2,4- and 3,4-dichlorophenyl, 3- and 4-trifluoromethylphenyl, methylphenyl, 3,4- and 2,5-dimethylphenyl, 4-isopropylphenyl, 3,5-di-tert.-butylphenyl, methoxyphenyl, 3,4-dimethoxyphenyl, 2,4,5- and 3,4,5-trimethoxyphenyl, hydroxyphenyl, 3,5-dihydroxyphenyl, 4-hydroxy-3,5-dimethyl-phenyl, 3-hydroxy-4-methoxy- and 4-hydroxy-3-methoxy-phenyl, 4-hydroxy-(3-methyl-5-tert.-butyl-, 2- and 4-acetylaminophenyl, 3,5-diisopropyl- and 3,5-di-tert.-butyl)phenyl, 4-carboxy- and 4-ethoxycarbonylphenyl, 4-cyanophenyl, 3-methoxycarbonylphenyl, 3-carboxy-5-methoxy-phenyl, 2-pyridinyl, 5-chloro-2-pyridinyl and 6-methyl-2-pyridinyl when X denotes ethenylene, or R_5 is different from phenyl, 4-methylphenyl, 4-methoxyphenyl, 4-bromophenyl and 2- and 4-chlorophenyl when X denotes 1,2-propylene attached to R_5 in 2-position, or R_5 is different from phenyl, 2- and 4-chlorophenyl and 3-methoxyphenyl when X denotes 1,2-propylene attached to R_5 in 1-position, or R_5 is different from 4-methoxyphenyl when X denotes 2,3-but-2-enylene or 1,2-but-1-enylene attached to R_5 in 2-position, or R_5 is different from 4-methoxyphenyl and 4-isopropyphenyl when X denotes 2,3-pent-2-enylene attached to R_5 in 3-position, or R_5 is different from phenyl, 4-methylphenyl, methoxyphenyl and 4-hydroxyphenyl when X denotes 3,4-hex-3-enylene;
b) in compounds of the formula I in which R_1 is methyl and R_2 and R_4 are hydrogen, R_5 is different from phenyl, 3-methylphenyl, 2-methoxyphenyl, 2-chlorophenyl, 4-cyanophenyl, , 2-pyridinyl and 6-methyl-2-pyridinyl when X denotes ethenylene;

c) in compounds of the formula I in which R_1 and R_2 are hydrogen and R_4 is carboxy, R_5 is different from phenyl, 3-methylphenyl, 4-methoxyphenyl and 4-bromophenyl when X denotes ethenylene;
d) in compounds of the formula I in which R_1 and R_2 are hydrogen and R_4 is methyl, R_5 is different from phenyl, 3-methoxy-, 4-methoxy- and 3,4-dimethoxyphenyl, 2-chloro- and 2,4-dichlorophenyl and 6-methyl-pyrid-2yl when X denotes ethenylene or R_5 is different from phenyl when X is 1,2-prop-1-enylene attached to R_5 in 2-position;
e) in compounds of the formula I wherein R_1 and R_2 are hydrogen and R_4 is 2-dimethylaminoethoxycarbonyl or 3-dimethylaminopropyloxycarbonyl, R_5 is different from 4-methoxyphenyl when X denotes ethenylene;
f) in compounds of the formula I in which R_1 and R_2 are hydrogen and R_4 is 2-dimethoxyethoxy, R_5 is different from phenyl, 4-methylphenyl and 4-methoxycarbonylphenyl when X denotes ethenylene;
g) R_5 is different from phenyl when R_1 and R_2 are hydrogen and R_4 is hydroxy or ethoxycarbonyl, or when R_1 and R_2 are hydrogen and R_4 is hydroxy, or when R_1 is methyl, R_2 is hydrogen and R_4 is methoxy, or R_1 is but-1-enyl, R_2 is hydrogen and R_4 is hydrogen, or R_1 is hydrogen and R_4 is 2-dimethoxyethoxy, and X is, in each case, ethenylene,
and provided that, when R_3 is hydrogen and X is ethynylene,
a') R_5 is different from phenyl, 2- and 4-nitrophenyl, 4-aminophenyl, 4-chlorophenyl, 4-methylphenyl, 4-methoxyphenyl, 4-ethoxycarbonylphenyl, 5-formyl-2-methoxy-phenyl, 5-carboxy-2-methyo-phenyl and pyridyl when R_1, R_2 and R_4 are hydrogen;
b') in compounds of the formula I in which R_2 and R_4 are hydrogen, R_5 is different from phenyl, 3-methylphenyl. 6-methylpyridin-2-yl and 2-methoxyphenyl when R_1 is methyl, R_5 is different form 6-bromopyridin-2-yl when R_1 is bromo, and R_5 is different form 6-hexyloxypyridin-2-yl when R_1 denotes hexyloxy;
c') in compounds of the formula I wherein R_1 and R_4 are hydrogen, R_5 is different from phenyl, 4-aminophenyl and 4-propylphenyl when R_2 is methyl, R_5 is different from phenyl, 4-cyanophenyl and 4-pentylphenyl when R_2 is ethyl, R_5 is different form 3-cyano-4-ethoxy-phenyland 3-bromo-4-methoxy-phenyl when R_2 is butyl, R_5 is different from 4-methoxyphenyl and 4 butyloxyphenyl when R_2 is pentyl, R_5 is different form 4-ter.-butylphenyl, 3-tert.-butyl-4-hydroxy-phenyl, 4-tert.-butyl-3-hydroxy-phenyl,and 4-hexyloxyphenyl when R_2 is carboxy, R_5 is different from phenyl when R_2 is methoxycarbonyl or methylcarbamoyl, R_4 is different form 3-tert.-butylphenyl, 3-tert.-butyl-4-hydroxy-phenyl and 4-(4-methylpentyl)phenyl when R_2 is ethoxycarbonyl, and R_5 is different from 4-pentyloxyphenyl when R_2 is 2-methylbutyloxycarbonyl;

d') in compounds of the formula I wherein R_1 and R_2 are hydrogen, R_5 is different from phenyl when R_4 is hydroxy, methyl, ethyl, carboxy, methoxycarbonyl or carbamoyl.

7. A compound according to claim 6, wherein

X represents an optionally halo-substituted (C_{2-4})alkenylene or alkynylene group bonded via vicinal unsaturated carbon atoms,

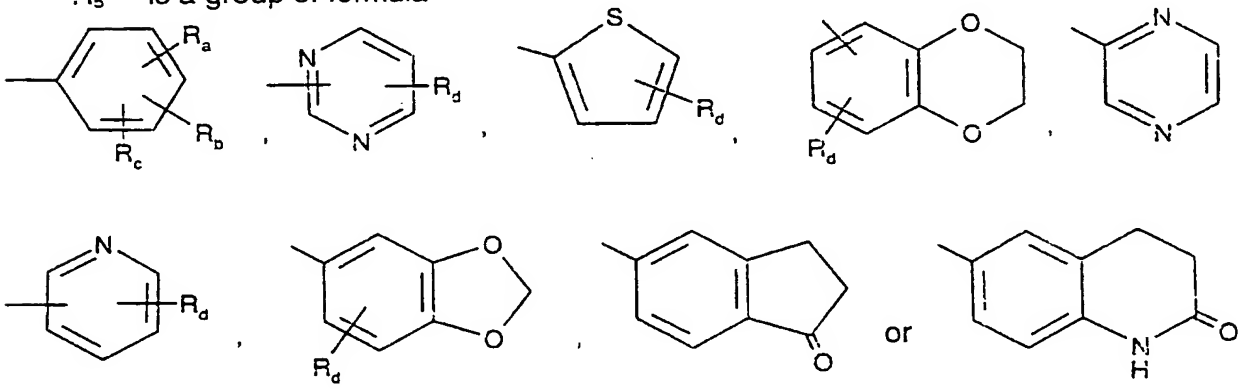
R_1 is hydrogen, (C_{1-4}) alkyl, (C_{1-4})alkoxy, hydroxy(C_{1-4})alkyl, cyano, ethynyl, carboxy, (C_{1-4})alkoxycarbonyl, di(C_{1-4})alkylamino, (C_{1-6})alkylaminocarbonyl, trifluoromethylphenylaminocarbonyl,

R_2 is hydrogen, hydroxy, (C_{1-4}) alkyl, hydroxy (C_{1-4}) alkyl, (C_{1-4}) alkoxy, carboxy, (C_{2-5})alkanoyloxy, (C_{1-4}) alkoxycarbonyl, di(C_{1-4})alkylamino(C_{1-4})alkanoyl, di(C_{1-4})alkylaminomethyl, 4-(4-fluoro-benzoyl)-piperidin-1-yl-carboxy, 4-t.-butyloxycarbonyl-piperazin-1-yl-carboxy, 4-(4-azido-2-hydroxybenzoyl)-piperazin-1-yl-carboxy or 4-(4-azido-2-hydroxy-3-iodo-benzoyl)-piperazin-1-yl-carboxy,

R_3 is hydrogen, (C_{1-4}) alkyl, carboxy, (C_{1-4})alkoxycarbonyl, (C_{1-4})alkylcarbamoyl, hydroxy(C_{1-4})alkyl, di(C_{1-4})alkylaminomethyl, morpholinocarbonyl or 4-(4-fluoro-benzoyl)-piperidin-1-yl-carboxy,

R_4 is hydrogen, hydroxy, (C_{1-4})alkoxy, carboxy, (C_{2-5})alkanoyloxy, (C_{1-4})alkoxycarbonyl, amino(C_{1-4})alkoxy, di(C_{1-4})alkylamino(C_{1-4})alkoxy, di(C_{1-4})alkylamino(C_{1-4})alkyl, carboxy (C_{1-4})alkylcarbonyl, (C_{1-4})alkoxycarbonyl-(C_{1-4})alkoxy, hydroxy(C_{1-4})alkyl, di(C_{1-4})alkylamino(C_{1-4})alkoxy, m-hydroxy-p-azidophenylcarbonylamino(C_{1-4})alkoxy, and

R_5 is a group of formula



wherein

R_a and R_b independently are hydrogen, hydroxy, halogen, nitro, cyano, carboxy, (C_{1-4})alkyl, (C_{1-4})alkoxy, hydroxy(C_{1-4})alkyl, (C_{1-4})alkoxycarbonyl, (C_{2-7})alkanoyl,

(C_{2-5})alkanoyloxy, (C_{2-5})alkanoyloxy(C_{1-4})alkyl, trifluoromethyl, trifluoromethoxy, trimethylsilylethynyl, (C_{2-5})alkynyl, amino, azido, amino (C_{1-4})alkoxy, (C_{2-5})alkanoylamino(C_{1-4})alkoxy, (C_{1-4})alkylamino(C_{1-4})alkoxy, di(C_{1-4})alkylamino(C_{1-4})alkoxy, (C_{1-4})alkylamino, di(C_{1-4})alkylamino, monohalobenzylamino, thienylmethylamino, thienylcarbonylamino, trifluoromethylphenylaminocarbonyl, tetrazolyl, (C_{2-5})alkanoylamino, benzylcarbonylamino, (C_{1-4})alkylamino-carbonylamino, (C_{1-4})alkoxycarbonyl-aminocarbonylamino or (C_{1-4})alkylsulfonyl,
R_c is hydrogen, fluorine, chlorine, bromine, hydroxy, (C_{1-4})alkyl, (C_{2-5})alkanoyloxy, (C_{1-4})alkoxy or cyano, and
R_d is hydrogen, halogen or (C_{1-4})alkyl.

8. A compound according to claim 6, wherein

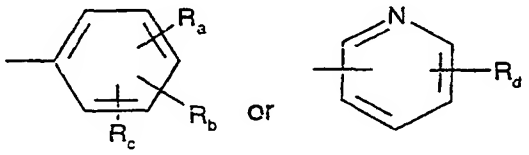
R_1 is hydrogen, (C_{1-4}) alkyl, (C_{1-4})alkoxy, cyano, ethynyl or di(C_{1-4})alkylamino,

R_2 is hydrogen, hydroxy, carboxy, (C_{1-4}) alkoxycarbonyl, di(C_{1-4})alkylaminomethyl, 4-(4-fluoro-benzoyl)-piperidin-1-yl-carboxy, 4-t.-butyloxycarbonyl-piperazin-1-yl-carboxy, 4-(4-azido-2-hydroxybenzoyl)-piperazin-1-yl-carboxy or 4-(4-azido-2-hydroxy-3-iodo-benzoyl)-piperazin-1-yl-carboxy,

R_3 is as defined in claim 7,

R_4 is hydrogen, hydroxy, carboxy, (C_{2-5})alkanoyloxy, (C_{1-4})alkoxycarbonyl, amino (C_{1-4})alkoxy, di(C_{1-4})alkylamino(C_{1-4})alkoxy, di(C_{1-4})alkylamino(C_{1-4})alkyl or hydroxy(C_{1-4})alkyl, and

R_5 is a group of formula



wherein
R_a and R_b independently are hydrogen, halogen, nitro, cyano, (C_{1-4})alkyl, (C_{1-4})alkoxy, trifluoromethyl, trifluoromethoxy or (C_{2-5})alkynyl, and
R_c and R_d are as defined in claim 7.

9. A compound according to claim 6, selected from

3-[2-(6-Methylpyridin-2-yl)-vinyl]-benzonitrile
2-[2-(6-Methyl-pyridin-2-yl)-vinyl]-benzonitrile

2-Methyl-6-[2-(pyridin-4-yl)-vinyl]-pyridine
2-Methyl-6-[2-(pyridin-3-yl)-vinyl]-pyridine
2-[2-(3-Bromophenyl)ethynyl]-6-methyl-pyridine
3-[2-(6-Methylpyridin-2-yl)ethynyl]-benzonitrile
2-Styryl-pyridin-3-ol
2-Methyl-6-[2-(3-nitro-phenyl)-vinyl]-pyridine
Acetic acid 6-[2-(2-chloro-phenyl)-vinyl]-pyridin-3-yl ester
6-[2-(2-Chloro-phenyl)-vinyl]-pyridin-3-ol
Acetic acid 2-[2-(2-chloro-phenyl)-vinyl]-pyridin-3-yl ester
2-[2-(2-Chloro-phenyl)-vinyl]-pyridin-3-ol
6-Methyl-2-styryl-pyridin-3-ol
Acetic acid 2-[2-(2-chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yl ester
2-[2-(2-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-ol
(Z)-6-Methyl-2-styryl-pyridin-3-ol
2-[2-(2-Nitro-phenyl)-vinyl]-pyridine
Acetic acid 2-[2-(4-chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yl ester
Acetic acid 6-[2-(4-chloro-phenyl)-vinyl]-2-methyl-pyridin-3-yl ester
2-[2-(4-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-ol
6-[2-(4-Chloro-phenyl)-vinyl]-2-methyl-pyridin-3-ol
Acetic acid 6-methyl-2-[2-(2-nitro-phenyl)-vinyl]-pyridin-3-yl ester
6-Methyl-2-[2-(2-nitro-phenyl)-vinyl]-pyridin-3-ol
Acetic acid 2-methyl-6-[2-(2-nitro-phenyl)-vinyl]-pyridin-3-yl ester
2-Methyl-6-[2-(2-nitro-phenyl)-vinyl]-pyridin-3-ol
Acetic acid 2-[2-(3-chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yl ester
Acetic acid 6-[2-(3-chloro-phenyl)-vinyl]-2-methyl-pyridin-3-yl ester
2-[2-(3-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-ol
6-[2-(3-Chloro-phenyl)-vinyl]-2-methyl-pyridin-3-ol
(Z)-(6-Styryl-pyridin-2-yl)-methanol
(E)-(6-Styryl-pyridin-2-yl)-methanol
Dimethyl-[3-(6-methyl-2-styryl-pyridin-3-yloxy)-propyl]-amine;
2-Methyl-6-styryl-pyridine 1-oxide
2-Styryl-pyridine 1-oxide
(E)-6-Methyl-2-(2-pyridin-2-yl-vinyl)-pyridin-3-ol
(Z)-6-Methyl-2-(2-pyridin-2-yl-vinyl)-pyridin-3-ol;
6-Styryl-pyridine-2-carbonitrile
2-[2-(2,6-Dichloro-phenyl)-vinyl]-6-methyl-pyridine

3-Methoxy-6-methyl-2-styryl-pyridine

6-Styryl-pyridine-2-carboxylic acid amide

2-[2-(6-Methyl-pyridin-2-yl)-vinyl]-benzonitrile

6-Styryl-pyridine-2-carboxylic acid;

6-Styryl-pyridine-2-carboxylic acid methyl ester

Acetic acid 2-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl ester

2-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenol

Acetic acid 2-methoxy-4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl ester

2-[2-(3-Chloro-phenyl)-vinyl]-6-methyl-pyridine

2-[2-(4-Chloro-phenyl)-vinyl]-6-methyl-pyridine

2-[2-(2-Chloro-phenyl)-vinyl]-5-ethyl-pyridine

1-(6-Styryl-pyridin-2-yl)-ethanone

6-[2-(2-Chloro-phenyl)-vinyl]-2-methyl-nicotinic acid ethyl ester

2-[2-(2-Chloro-phenyl)-vinyl]-6-methyl-nicotinic acid ethyl ester

2-[2-(6-Methyl-pyridin-2-yl)-vinyl]-benzoic acid;

3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-benzoic acid

4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-benzoic acid

3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-benzoic acid methyl ester

4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-benzoic acid methyl ester

2-Methoxy-4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenol

{3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-methanol;

6-Styryl-pyridine-2-carboxylic acid .tert.-butylamide

2-(2-Bromo-2-phenyl-vinyl)-6-methyl-pyridine;

6-Styryl-pyridine-2-carboxylic acid hexylamide;

6-[2-(2-Chloro-phenyl)-vinyl]-2-methyl-nicotinic acid

2-[2-(2-Chloro-phenyl)-vinyl]-6-methyl-nicotinic acid

2-[2-(3,5-Dichloro-phenyl)-vinyl]-6-methyl-pyridine

2-Methyl-6-[2-(3-trifluoromethyl-phenyl)-vinyl]-pyridine

(E)-6-[2-(4-Pyridyl)vinyl]-2-picoline

N,N-Diethyl-3-[2-(6-methyl-pyridin-2-yl)-vinyl]-benzamide;

N,N-Diethyl-4-[2-(6-methyl-pyridin-2-yl)-vinyl]-benzamide;

(E)-6-[2-(3-pyridyl)vinyl]-2-Picoline

{2-[2-(2-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-acetic acid ethyl ester

3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-.N.-(3-trifluoromethyl-phenyl)-benzamide;

4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-.N.-(3-trifluoromethyl-phenyl)-benzamide

2-[2-(3-Nitro-phenyl)-vinyl]-pyridine

6-Styryl-pyridine-2-carboxylic acid (3-trifluoromethyl-phenyl)-amide

2-(6-Styryl-pyridin-2-yl)-propan-2-ol

2-Methyl-6-(2-thiophen-2-yl-vinyl)-pyridine

2-[2-(3-Cyano-phenyl)-vinyl]-pyridine

2-[2-(3-Bromo-phenyl)-vinyl]-6-methyl-pyridine

2-[2-(3-Bromo-phenyl)-2-fluoro-vinyl]-6-methyl-pyridine

2-[2-(3,5-Dimethylphenyl)-2-fluoro-vinyl]-6-methyl-pyridine

2-[2-(2,3-Dimethoxy-phenyl)-vinyl]-6-methyl-pyridine

2-[2-(2,3-Dichloro-phenyl)-vinyl]-6-methyl-pyridine

2-[2-(3-Chloro-phenyl)-1-methyl-vinyl]-pyridine

{2-[2-(2-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yl}-methanol

2-Methyl-6-[2-(3-trimethylsilanylethynyl-phenyl)-vinyl]-pyridine

2-[2-(3,4-Difluoro-phenyl)-vinyl]-6-methyl-pyridine

2-[2-(3-Ethynyl-phenyl)-vinyl]-6-methyl-pyridine

2-[2-(3,5-Difluoro-phenyl)-vinyl]-6-methyl-pyridine

2-[2-(3-Fluoro-phenyl)-vinyl]-6-methyl-pyridine

2-[2-(3-Methoxy-phenyl)-vinyl]-6-methyl-pyridine

2-Methyl-6-[2-(3-phenoxy-phenyl)-vinyl]-pyridine

2-[2-(3-Benzyloxy-phenyl)-vinyl]-6-methyl-pyridine

2-[2-(2,5-Difluoro-phenyl)-vinyl]-6-methyl-pyridine

{2-[2-(2-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-acetic acid

(3-{2-[2-(3-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethyl-amine

{6-[2-(2-Chloro-phenyl)-vinyl]-2-methyl-pyridin-3-yl}-methanol

2-(3-Bromo-phenylethynyl)-6-methyl-pyridine

2-Methyl-6-{2-[3-(3-trifluoromethyl-phenoxy)-phenyl]-vinyl}-pyridine

2-[2-(3,5-Dimethoxy-phenyl)-vinyl]-6-methyl-pyridine

2-[2-(3-Chloro-phenyl)-vinyl]-3-methoxy-6-methyl-pyridine

Acetic acid 4-bromo-2-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl ester

Acetic acid 3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl ester

2-[2-(3,4-Dichloro-phenyl)-vinyl]-6-methyl-pyridine

4-Bromo-2-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenol

Acetic acid 2-[2-(3,5-dichloro-phenyl)-vinyl]-6-methyl-pyridin-3-yl ester

Acetic acid 6-[2-(3,5-dichloro-phenyl)-vinyl]-2-methyl-pyridin-3-yl ester

Acetic acid 2-[2-(3,5-dichloro-phenyl)-vinyl]-pyridin-3-yl ester

2-Methyl-6-(2-naphthalen-1-yl-vinyl)-pyridine

2-[2-(2,3-Dihydro-benzo[1,4]dioxin-6-yl)-vinyl]-6-methyl-pyridine

2-Methyl-6-(2-naphthalen-2-yl-vinyl)-pyridine

2-{2-[3-(3,5-Dichloro-phenoxy)-phenyl]-vinyl}-6-methyl-pyridine

2-[2-(3-Chloro-phenyl)-propenyl]-6-methyl-pyridine

2-[2-(2,3-Dihydro-benzofuran-5-yl)-vinyl]-6-methyl-pyridine

2-[2-(4-Fluoro-phenyl)-vinyl]-6-methyl-pyridine

2-Methyl-6-(2-o-tolyl-vinyl)-pyridine

2-Methyl-6-(2-p-tolyl-vinyl)-pyridine

2-Methyl-6-(2-p-tolyl-propenyl)-pyridine

3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenylamine

(2,3-Dimethoxy-7-nitro-quinoxalin-5-ylmethyl)-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-amine

N-{3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-acetamide

N-{3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-2-phenyl-acetamide

2,2-Dimethyl-N-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-propionamide

Thiophene-2-carboxylic acid {3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-amide

Cyclohexanecarboxylic acid {3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-amide

1-(4-Bromo-phenyl)-3-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-urea

2-Methyl-6-[2-(4-nitro-phenyl)-vinyl]-pyridine

4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenylamine

2-[2-(3,5-Dichloro-phenyl)-vinyl]-6-methyl-pyridin-3-ol

6-[2-(3,5-Dichloro-phenyl)-vinyl]-2-methyl-pyridin-3-ol

2-[2-(3,5-Dichloro-phenyl)-vinyl]-pyridin-3-ol

2-[2-(6-Chloro-benzo[1,3]dioxol-5-yl)-vinyl]-6-methyl-pyridine

2-[2-(2,3-Difluoro-phenyl)-vinyl]-6-methyl-pyridine

2-[2-(3,4-Dichloro-phenyl)-propenyl]-6-methyl-pyridine

2-[2-(3,5-Bis-trifluoromethyl-phenyl)-vinyl]-6-methyl-pyridine

Acetic acid 2-methoxy-6-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl ester

2-Methoxy-6-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenol

2-Methyl-6-[2-(2,3,6-trifluoro-phenyl)-vinyl]-pyridine

2-[2-(4-Fluoro-3-trifluoromethyl-phenyl)-vinyl]-6-methyl-pyridine

2-Methyl-6-(2,3,6-trifluoro-phenylethynyl)-pyridine

Acetic acid 4-chloro-2-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl ester

Acetic acid 2,6-di-.tert.-butyl-4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl ester

3-(6-Methyl-pyridin-2-ylethynyl)-benzamide

Acetic acid 4-bromo-2-methoxy-6-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl ester

2-(6-Chloro-benzo[1,3]dioxol-5-ylethynyl)-6-methyl-pyridine

2-[2-(3,5-Dichloro-phenyl)-vinyl]-3-methoxy-6-methyl-pyridine
2-[2-(3,5-Dichloro-phenyl)-vinyl]-3-methoxy-pyridine
5-Azido-2-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenol
2-[2-(Pyridin-3-yl)ethynyl]-6-methyl-pyridine
N-{3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-succinamic acid
1-tert.-Butyl-3-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-urea
5-({3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenylamino}-methyl)-7-nitro-1,4-dihydro-quinoxaline-2,3-dione
Tetrahydro-furan-2-carboxylic acid {3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-amide
(1-{3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenylcarbamoyl}-2-phenyl-ethyl)-carbamic acid tert.-butyl ester
({3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenylcarbamoyl}-methyl)-carbamic acid tert.-butyl ester
Diethyl-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-amine
Ethyl-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-amine
Ethyl-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-amine
2-(2-Ethoxy-3,6-difluoro-phenylethynyl)-6-methyl-pyridine
2-(3,5-Difluoro-phenylethynyl)-6-methyl-pyridine
2-(3-Fluoro-phenylethynyl)-6-methyl-pyridine
2-[2-(3,5-Dimethyl-phenyl)-vinyl]-6-methyl-pyridine
2-[2-(3,4-Dimethoxy-phenyl)-vinyl]-6-methyl-pyridine
2-(3,4-Dichloro-phenylethynyl)-6-methyl-pyridine
2-(4-Ethoxy-3-trifluoromethyl-phenylethynyl)-6-methyl-pyridine
2-(4-Fluoro-phenylethynyl)-6-methyl-pyridine
2-Methyl-6-o-tolylethynyl-pyridine
2-(3,4-Difluoro-phenylethynyl)-6-methyl-pyridine
2-Methyl-6-[2-(2,3,5-trichloro-phenyl)-vinyl]-pyridine
1-[3-(6-Methyl-pyridin-2-ylethynyl)-phenyl]-ethanone
2-Methyl-6-(3-trifluoromethyl-phenylethynyl)-pyridine
2-Methyl-6-(3-nitro-phenylethynyl)-pyridine
6-[2-(3,5-Dichloro-phenyl)-vinyl]-3-methoxy-2-methyl-pyridine
{2-[2-(2-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yl}-morpholin-4-yl-methanone
(3-{2-[2-(3,5-Dichloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethyl-amine
N-{4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-succinamic acid
N-{4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-2-phenyl-acetamide
({4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenylcarbamoyl}-methyl)-carbamic acid .tert.-butyl ester
1-(tert.-Butyl-3-{4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-urea

{3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-thiophen-2-ylmethyl-amine hydrochloride salt
Cyclohexylmethyl-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-amine hydrochloride salt
{4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-thiophen-2-ylmethyl-amine
Cyclohexylmethyl-{4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-amine
2-Amino-N-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-3-phenyl-propionamide
2-Amino-N-{3-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-acetamide
2-Amino-N-{4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-acetamide
1-[1-({2-[2-(2-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-acetyl)-piperidin-4-yl]-imidazolidin-2-one
(1-{4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenylamino}-ethyl)-phosphonic acid dimethyl ester
2-(3-Ethoxy-4-fluoro-phenylethynyl)-6-methyl-pyridine
2-(3-Chloro-phenylethynyl)-6-methyl-pyridine
1-(3-Pyridin-2-ylethynyl-phenyl)-ethanone
4-Chloro-2-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenol
4-Bromo-2-methoxy-6-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenol
2-(2,5-Difluoro-phenylethynyl)-6-methyl-pyridine
2-(3,5-Dimethyl-phenylethynyl)-6-methyl-pyridine
2-[2-(3,5-Dibromo-phenyl)-vinyl]-6-methyl-pyridine
3-(6-Methyl-pyridin-2-ylethynyl)-benzonitrile
2-Methyl-6-[2-(pyrimidin-5-yl)-ethynyl]-pyridine
(2-{2-[2-(3-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-ethyl)-dimethyl-amine
Acetic acid 1-{4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenyl}-ethyl ester
3-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenol
3-(6-Methyl-pyridin-2-ylethynyl)-phenylamine
.N.-[3-(6-Methyl-pyridin-2-ylethynyl)-phenyl]-2-phenyl-acetamide
Thiophene-2-carboxylic acid [3-(6-methyl-pyridin-2-ylethynyl)-phenyl]-amide
2-Methyl-6-thiophen-2-ylethynyl-pyridine
3-(6-Methyl-pyridin-2-ylethynyl)-benzoic acid ethyl ester
2-(3,5-Dibromo-phenylethynyl)-6-methyl-pyridine
{2-[2-(2-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-ylmethyl}-dimethyl-amine
(3-{6-[2-(3-Chloro-phenyl)-vinyl]-2-methyl-pyridin-3-yloxy}-propyl)-dimethyl-
5-Azido-4-iodo-2-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenol
2,6-Di-tert.-butyl-4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenol
1-{4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-ethanol
2-Methyl-6-[2-(pyrimidin-2-yl)-ethynyl]-pyridine
[3-(6-Methyl-pyridin-2-ylethynyl)-phenyl]-phenyl-methanone

6-(6-Methyl-pyridin-2-ylethynyl)-3,4-dihydro-1H-quinolin-2-one

2-(3-{2-[2-(3-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-isoindole-1,3-dione

3-Methoxy-6-methyl-2-.m.-tolylethynyl-pyridine

Acetic acid 2-[2-(6-methyl-pyridin-2-yl)-vinyl]-4-nitro-phenyl ester

6-(6-Methyl-pyridin-2-ylethynyl)-indan-1-one

2-Methyl-6-[2-(pyrazin-2-yl)-ethynyl]-pyridine

N-Methyl-.N.-(3-{4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenoxy}-propyl)-acetamide

2-[2-(3,5-Bis-trifluoromethyl-phenyl)-1-ethoxy-vinyl]-6-methyl-pyridine

Acetic acid 2-phenylethynyl-pyridin-3-yl ester

Acetic acid 6-methyl-2-m-tolylethynyl-pyridin-3-yl ester

Acetic acid 4-[2-(6-methyl-pyridin-2-yl)-vinyl]-2-nitro-phenyl ester

2-[2-(6-Methyl-pyridin-2-yl)-vinyl]-4-nitro-phenol

Dimethyl-[3-(2-phenylethynyl-pyridin-3-yloxy)-propyl]-amine

Dimethyl-(3-{4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenoxy}-propyl)-amine

1-{4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-phenyl}-ethanone

2-(3-Fluoro-phenylethynyl)-quinoline

Acetic acid 2-methyl-6-styryl-pyridin-3-yl ester

4-[2-(6-Methyl-pyridin-2-yl)-vinyl]-2-nitro-phenol

3-Ethoxy-4-[2-(6-methyl-pyridin-2-yl)-vinyl]-2-nitro-phenol

4-(6-Methyl-pyridin-2-ylethynyl)-2-nitro-phenol

Acetic acid 2-[2-(6-methyl-pyridin-2-yl)-vinyl]-6-nitro-phenyl ester

Dimethyl-[3-(6-methyl-2-phenylethynyl-pyridin-3-yloxy)-propyl]-amine

2-Azido-4-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenol

Dimethyl-[3-(6-methyl-2-.m.-tolylethynyl-pyridin-3-yloxy)-propyl]-amine

2-(3-Methanesulfonyl-phenylethynyl)-6-methyl-pyridine

3-{2-[2-(3-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propylamine

4-Azido-N-(3-{2-[2-(3-chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-2-hydroxy-benzamide

3-[3-(3-Dimethylamino-propoxy)-6-methyl-pyridin-2-ylethynyl]-benzonitrile

5-(6-Methyl-pyridin-2-ylethynyl)-indan-1-one

2-Methyl-6-(2,3,5-trichloro-phenylethynyl)-pyridine

2-[2-(6-methyl-pyridin-3-yl)ethynyl]-6-methyl-pyridine

Dimethyl-{3-[6-methyl-2-(3-trifluoromethyl-phenylethynyl)-pyridin-3-yloxy]-propyl}-amine

2-[2-(6-methyl-pyridin-3-yl)ethynyl]-3-methoxy 6-methyl-pyridine hydrochloride salt

2-Methyl-6-(5,6,7,8-tetrahydro-naphthalen-2-ylethynyl)-pyridine

3-[2-(3-Chloro-phenylethynyl)-6-methyl-pyridin-3-yloxy]-propylamine

(3-{4-Bromo-2-methoxy-6-[2-(6-methyl-pyridin-2-yl)-vinyl]-phenoxy}-propyl)-dimethyl-amine;

[6-(3-Fluoro-phenylethynyl)-pyridin-2-yl]-dimethyl-amine

6'-(3-Fluoro-phenylethynyl)-3,4,5,6-tetrahydro-2H-[1,2']bipyridinyl

{3-[2-(3-Chloro-phenylethynyl)-6-methyl-pyridin-3-yloxy]-propyl}-dimethyl-amine

4-Azido-N-{3-[2-(3-chloro-phenylethynyl)-6-methyl-pyridin-3-yloxy]-propyl}-2-hydroxy-benzamide

1-[3-(6-Methyl-pyridin-2-ylethynyl)-phenyl]-1H-[1,2,4]triazole-3-carboxylic acid ethyl ester

1-[3-(6-Methyl-2-phenylethynyl-pyridin-3-yloxy)-propyl]-piperidin-3-ol

2-Ethynyl-6-(3-fluoro-phenylethynyl)-pyridine

3-Methyl-6-(6-methyl-pyridin-2-ylethynyl)-3H-benzooxazol-2-one

1-[3-(6-Methyl-pyridin-2-ylethynyl)-phenyl]-1H-[1,2,4]triazole-3-carboxylic acid

1-[3-(6-Methyl-pyridin-2-ylethynyl)-phenyl]-1H-[1,2,4]triazole-3-carboxylic acid dimethylamide

1-[3-(6-Methyl-2-phenylethynyl-pyridin-3-yloxy)-propyl]-piperidin-4-ol

5-(6-Methyl-pyridin-2-ylethynyl)-2-nitro-phenol

5-[2-Bromo-2-(6-methyl-pyridin-2-yl)-vinyl]-2-nitro-phenol

5-[2-(6-Methyl-pyridin-2-yl)-E-vinyl]-2-nitro-phenol

5-[2-(6-Methyl-pyridin-2-yl)-Z-vinyl]-2-nitro-phenol

4-Azido-2-hydroxy-N-[3-(6-methyl-pyridin-2-ylethynyl)-phenyl]-benzamide

5-(3-Dimethylamino-propoxy)-6-phenylethynyl-pyridine-2-carboxylic acid ethyl ester

6-Methyl-2-styryl-pyrimidin-4-ol

2-Ethyl-6-(3-fluoro-phenylethynyl)-pyridine

2-(3,5-Dichloro-phenylethynyl)-6-methyl-pyridine

2-Methyl-6-(3-trifluoromethoxy-phenylethynyl)-pyridine

2-Methyl-6-(3-[1,2,4]triazol-1-yl-phenylethynyl)-pyridine

4-(6-Methyl-pyridin-2-ylethynyl)-phthalonitrile

2-Methyl-6-{2-[3-(1H-tetrazol-5-yl)-phenyl]-vinyl}-pyridine; compound with formic acid

3-[2-(3,5-Dichloro-phenylethynyl)-6-methyl-pyridin-3-yloxy]-propylamine

{3-[2-(3,5-Dichloro-phenylethynyl)-6-methyl-pyridin-3-yloxy]-propyl}-dimethyl-amine

2-(3,5-Dimethyl-phenylethynyl)-3-methoxy-6-methyl-pyridine

2-[2-(3-Fluoro-phenyl)-vinyl]-6-methyl-pyridin-3-ol

6-(3-Fluoro-phenylethynyl)-2-methyl-nicotinic acid ethyl ester

2-Azido-5-(6-methyl-pyridin-2-ylethynyl)-phenol

6-(3,4-Dimethoxy-phenylethynyl)-5-(3-dimethylamino-propoxy)-pyridine-2-carboxylic acid ethyl ester

2-(4-Methoxy-3-trifluoromethyl-phenylethynyl)-6-methyl-pyridine

2-(3-Fluoro-phenylethynyl)-6-methoxy-pyridine

2-(3-Fluoro-phenylethynyl)-5-methyl-pyridine
6-(3,5-Dichloro-phenylethynyl)-5-(3-dimethylamino-propoxy)-pyridine-2-carboxylic acid ethyl ester
5-(3-Dimethylamino-propoxy)-6-(3,5-dimethyl-phenylethynyl)-pyridine-2-carboxylic acid ethyl ester
6-(3-Fluoro-phenylethynyl)-2-methyl-nicotinic acid
[6-(3-Fluoro-phenylethynyl)-2-methyl-pyridin-3-yl]-methanol
[4-(4-Fluoro-benzoyl)-piperidin-1-yl]-[6-(3-fluoro-phenylethynyl)-2-methyl-pyridin-3-yl]-methanone
2-(3-Fluoro-phenylethynyl)-6-methyl-nicotinic acid ethyl ester
2-(3-Fluoro-phenylethynyl)-4,6-dimethyl-pyridine
6-(3-Fluoro-phenylethynyl)-.N.-(5-methoxy-indan-2-ylmethyl)-2-methyl-nicotinamide
{[6-(3-Fluoro-phenylethynyl)-2-methyl-pyridine-3-carbonyl]-amino}-phenyl-acetic acid methyl ester
2-Methyl-6-(5-methyl-thiophen-2-ylethynyl)-pyridine
2-Methyl-6-(2,3,5-trimethyl-phenylethynyl)-pyridine
3-{2-[2-(3-Fluoro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propan-1-ol
[6-(3-Fluoro-phenylethynyl)-2-methyl-pyridin-3-ylmethyl]-dimethyl-amine
2,2-Dimethyl-propionic acid 3-[2-(3-fluoro-phenylethynyl)-6-methyl-pyridin-3-yloxy]-propyl ester
2-Azido-4-iodo-5-(6-methyl-pyridin-2-ylethynyl)-phenol
6-Azido-2,4-diiodo-3-(6-methyl-pyridin-2-ylethynyl)-phenol
4-Azido-2-hydroxy-5-iodo-.N.-[3-(6-methyl-pyridin-2-ylethynyl)-phenyl]-benzamide
Acetic acid 3-acetoxymethyl-5-(6-methyl-pyridin-2-ylethynyl)-benzyl ester
(Benzyl-{[2-(3-fluoro-phenylethynyl)-6-methyl-pyridin-3-yloxy]-acetyl}-amino)-acetic acid ethyl ester
2-[2-(3-Fluoro-phenyl)-vinyl]-6-methyl-isonicotinic acid ethyl ester
3-[2-(3-Fluoro-phenylethynyl)-6-methyl-pyridin-3-yloxy]-propan-1-ol
[3-Hydroxymethyl-5-(6-methyl-pyridin-2-ylethynyl)-phenyl]-methanol
(3-{2-[2-(3,5-Dimethyl-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethyl-amine
[4-(4-Fluoro-benzoyl)-piperidin-1-yl]-{6-[2-(3-fluoro-phenyl)-vinyl]-2-methyl-pyridin-3-yl}-methanone
2-[2-(3-Fluoro-phenyl)-vinyl]-6-methyl-isonicotinic acid
{6-[2-(2-Chloro-phenyl)-vinyl]-2-methyl-pyridin-3-yl}-[4-(4-fluoro-benzoyl)-piperidin-1-yl]-methanone
2-(3-Ethynyl-phenylethynyl)-6-methyl-pyridine

(3-{2-[2-(2,6-Dichloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethyl-amine
(3-{2-[2-(2,3-Dichloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethyl-amine
4-[6-(3-Fluoro-phenylethynyl)-2-methyl-pyridine-3-carbonyl]-piperazine-1-carboxylic acid tert.-butyl ester
[6-(3-Fluoro-phenylethynyl)-2-methyl-pyridin-3-yl]-piperazin-1-yl-methanone
[4-(4-Azido-2-hydroxy-benzoyl)-piperazin-1-yl]-[6-(3-fluoro-phenylethynyl)-2-methyl-pyridin-3-yl]-methanone
(3-{2-[2-(2,4-Dichloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethyl-amine
2-(3-Fluoro-phenylethynyl)-6-methyl-isonicotinic acid ethyl ester
2-(3-Fluoro-phenylethynyl)-6-methyl-isonicotinic acid .tert.-butyl ester
2-(3-Fluoro-phenylethynyl)-6-methyl-isonicotinic acid
[2-(3-Fluoro-phenylethynyl)-6-methyl-pyridin-4-yl]-methanol
[4-(4-Fluoro-benzoyl)-piperidin-1-yl]-[2-(3-fluoro-phenylethynyl)-6-methyl-pyridin-4-yl]-methanone
3-Allyloxy-2-[2-(3,5-dichloro-phenyl)-vinyl]-6-methyl-pyridine
[2-(3-Fluoro-phenylethynyl)-6-methyl-pyridin-4-yl]-morpholin-4-yl-methanone
Acetic acid 3-(6-methyl-pyridin-2-ylethynyl)-benzyl ester
[2-(3-Fluoro-phenylethynyl)-6-methyl-pyridin-4-ylmethyl]-dimethyl-amine
(3-{2-[2-(3,5-Dichloro-phenyl)-propenyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethyl-amine
2-(3-Fluoro-phenylethynyl)-3-methoxy-6-methyl-pyridine
(3-{2-[2-(3,5-Dichloro-phenyl)-vinyl]-pyridin-3-yloxy}-propyl)-dimethyl-amine
(4-Azido-2-hydroxy-5-iodo-phenyl)-{4-[6-(3-fluoro-phenylethynyl)-2-methyl-pyridine-3-carbonyl]-piperazin-1-yl}-methanone
4-Azido-N-{3-[2-(3-chloro-phenylethynyl)-6-methyl-pyridin-3-yloxy]-propyl}-2-hydroxy-5-iodo-benzamide
4-(2-Pyridin-2-yl-vinyl)-benzoic acid ethyl ester
(3-{2-[2-(4-Chloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethyl-amine
[3-(6-Methyl-pyridin-2-ylethynyl)-phenyl]-methanol
6-(3-Fluoro-phenylethynyl)-nicotinic acid tert.-butyl ester
(3-{2-[2-(3,4-Dichloro-phenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethyl-amine
2-(1-Bromo-2-phenyl-vinyl)-4-methyl-pyrimidine
6-(3-Fluoro-phenylethynyl)-nicotinic acid
[4-(4-Fluoro-benzoyl)-piperidin-1-yl]-[6-(3-fluoro-phenylethynyl)-pyridin-3-yl]-methanone
2-(2-.tert.-Butoxy-3,6-difluoro-phenylethynyl)-6-methyl-pyridine
2-Methyl-6-[2-(2,4,5-trifluoro-phenyl)-vinyl]-pyridine
2-Methyl-6-[2-(2,3,4-trifluoro-phenyl)-vinyl]-pyridine

3-(6-Methyl-pyridin-2-ylethynyl)-phenol
2-Methyl-6-[2-(3,4,5-trifluoro-phenyl)-vinyl]-pyridine
2-(3-Methoxy-phenylethynyl)-6-methyl-pyridine
2-Methyl-6-(2,3,4-trifluoro-phenylethynyl)-pyridine
and pharmaceutically acceptable salts thereof.

10. (3-{2-[2-trans-(3,5-dichlorophenyl)-vinyl]-6-methyl-pyridin-3-yloxy}-propyl)-dimethylamine in free form or in form of a pharmaceutically acceptable salt.

11. A pharmaceutical composition comprising as pharmaceutical active ingredient, together with customary pharmaceutical excipients, a compound according to any of claims 6 to 10, in free form or in form of a pharmaceutically acceptable salt.

12. A method of treating disorders mediated full or in part by mGluR1 or mGluR5, which method comprises administering to a warm-blooded organism in need of such treatment a therapeutically effective amount of an 2-arylalkenyl- , 2-heteroarylalkenyl-, 2-arylalkynyl-, 2-heteroarylalkynyl-, 2-arylazo- and 2-heteroarylazo- pyridine or a pharmaceutically acceptable salt thereof.

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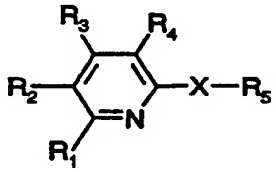

(I)

(57) Abstract

Compounds of the formula (I), wherein X and R_1 to R_5 are as defined in the description, are useful for treating disorders mediated full or in part by mGluR5.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/04266

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D213/65 A61K31/44 C07D213/80 C07D401/12 C07D401/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 334 119 A (BOEHRINGER INGELHEIM PHARMA) 27 September 1989 see page 16; claim 1 ---	1-3,6-11
X	DOWELL R.I.; HALES, N. H., TUCKER H.: "Novel inhibitors of prolyl 4-hydroxylase. Part 4. Pyridine-2-carboxylic acid analogues with alternative 2-substituents" EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, vol. 28, no. 6, 1993, pages 513-516, XP002087215 see page 514; example 19 --- -/--	1-3,6-11

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 December 1998	07. 01. 99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lauro, P

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LAZER E. S. ET AL: "Effect of structure on potency and selectivity in 2,6-disubstituted 4-(2-arylethenyl)phenol lipoxygenase inhibitors" JOURNAL OF MEDICINAL CHEMISTRY, vol. 33, no. 7, 1990, pages 1892-98, XP002087216 see page 1894; example 54 ---	1-3,6-11
X	BAHNER, C. T. ET AL. : "Di- and tri-methoxystyryl derivatives of heterocyclic nitrogen compounds" ARZNEIM. FORSCH. / DRUG RES., vol. 31, no. 3, 1981, pages 404-6, XP002087217 see page 405; examples 6-8 ---	1-3,6-11
X	HONMA Y; HANAMOTO K; HASHIYAMA T; SEKINE Y; TAKEDA M; ONO Y: "Antiallergic agents. 3. N-(1H-tetrazol-5-yl)-2-pyridinecarboxamides" JOURNAL OF MEDICINAL CHEMISTRY, vol. 27, no. 2, 1984, pages 125-128, XP002087218 see example 9 ---	1-3,6-11
X	MORI M ET AL: "THE NEMATICIDAL ACTIVITY OF ACETYLENE COMPOUNDS" AGRICULTURAL AND BIOLOGICAL CHEMISTRY, vol. 46, no. 1, 1982, pages 309-311, XP000645051 see example 14; table III ---	1-3,6-10
X	D. JERCHEL; H. E. HECK: "Kondensation von Methylpyridinen mit Benzaldehyd" JUSTUS LIEBIGS ANN. CHEM., vol. 613, 1958, pages 171-177, XP002087219 see page 174; example III ---	1-3,6-10
X	SADAO ARAI ET AL.: "Synthesis and reactions of methylbenzo[c]quinolizinium salts" JOURNAL OF HETEROCYCLIC CHEMISTRY, XP002087220 see example 4 ---	1-3,6-10
X	B.D. SHAW; E.A. WAGSTAFF: "The nitration of beta-phenylethylpyridines and related compounds" JOURNAL OF THE CHEMICAL SOCIETY, XP002087221 * see compounds of formula (II) and (III)* --- -/--	1-3,6-10

2

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 19049 A (CIBA GEIGY AG ;SANDOZ AG (DE); NOVARTIS ERFINDUNGEN VERWALTUN (AT)) 29 May 1997 see page 1-5 ---	1-3,5-11
A	WO 97 05109 A (NOVONORDISK AS ;LUNDBECK JANE MARIE (DK); KANSTRUP ANDERS (DK)) 13 February 1997 see page 13-17; claim 1 -----	1-3,5-11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 98/04266

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 4,12
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 4 and 12 are directed to a method for treatment of the human/animal body by therapy, the search has been carried out and based on the alleged effects of the compounds/compositions

2. [X] Claims Nos.: -
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 98/04266

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0334119 A	27-09-1989	AU 628324 B	17-09-1992
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		EP 0843660 A	27-05-1998
		US 5696148 A	09-12-1997

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

In view of the huge number of documents which disclose the compounds claimed in claims 1-3, 6-11, and which could not all be cited in the search report, the search is to be considered incomplete as far as the claims directed to compounds per se and their pharmaceutical compositions are concerned. The compounds in the form of photoaffinity ligands and radioactive markers have not been searched since no support in the description could be found. Claim 5 has been searched completely.